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Continuity Information for 09/284233

Parent Data

09284233

is a national stage entry of PCT/EP97/04744 International Filing Date: **09/01/1997**

Child Data

09976297 **is a continuation in part of** 09284233

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Foreign Information for 09/284233

Priority#	Date	Country
96116337.5	10/11/1996	EUROPEAN PATENT OFFICE (EPO)

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NiceProt View of Swiss-Prot: Q9ZMZ4

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[Entry info] [Name and origin] [References] [Comments] [Cross-references] [Keywords] [Features] [Sequence] [Tools]

Note: most headings are clickable, even if they don't appear as links. They link to the user manual or other documents.

Entry information

Entry name	**UREA_HELPJ**
Primary accession number	**Q9ZMZ4**
Secondary accession numbers	None
Entered in Swiss-Prot in	Release 39, May 2000
Sequence was last modified in	Release 39, May 2000
Annotations were last modified in	Release 43, March 2004

Name and origin of the protein

Protein name	**Urease alpha subunit**
Synonyms	**EC 3.5.1.5** **Urea amidohydrolase alpha subunit**
Gene name	**UREA** or **HPUA** or **JHP0068**
From	Helicobacter pylori J99 (Campylobacter pylori J99) [TaxID: 85963]
Taxonomy	Bacteria; Proteobacteria; Epsilonproteobacteria; Campylobacterales; Helicobacteraceae; Helicobacter.

References

[1] SEQUENCE FROM NUCLEIC ACID.
MEDLINE=99120557; PubMed=9923682; [NCBI, ExPASy, EBI, Israel, Japan]
Alm R.A., Ling L.-S.L., Moir D.T., King B.L., Brown E.D., Doig P.C., Smith D.R., Noonan B., Guild B.C., deJonge B.L., Carmel G., Tummino P.J., Caruso A., Uria-Nickelsen M., Mills D.M., Ives C., Gibson R., Merberg D., Mills S.D., Jiang Q., Taylor D.E., Vovis G.F., Trust T.J.;
"Genomic sequence comparison of two unrelated isolates of the human gastric pathogen Helicobacter pylori.";
Nature 397:176-180(1999).

Comments

- ***CATALYTIC ACTIVITY***: Urea + H_2O = CO_2 + 2 NH_3.
- ***SUBUNIT***: Heterodimer of an alpha subunit and a beta subunit *(By similarity)*.

- ***SUBCELLULAR LOCATION***: Cytoplasmic *(By similarity)*.
- ***SIMILARITY***: In the N-terminal section; belongs to the urease gamma subunit family.
- ***SIMILARITY***: In the C-terminal section; belongs to the urease beta subunit family.
- ***CAUTION***: In Helicobacter the alpha subunit is what is known, in other bacteria as the beta subunit.

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Cross-references

EMBL	AE001446; AAD05652.1; -.[EMBL / GenBank / DDBJ] [CoDingSequence]
PIR	B71977; B71977.
HSSP	P14916; 1E9Z. [HSSP ENTRY / PDB]
CMR	Q9ZMZ4; JHP0068.
HAMAP	MF_00739; fused; 1. PBIL [Family / Alignment / Tree]
InterPro	IPR002019; Urease_beta. IPR002026; Urease_gamma. IPR008223; Urease_gammabeta. Graphical view of domain structure.
Pfam	PF00699; Urease_beta; 1. PF00547; urease_gamma; 1. Pfam graphical view of domain structure.
PIRSF	PIRSF001225; Urease_gammabeta; 1.
ProDom	PD002326; Urease_beta; 1. PD002319; Urease_gamma; 1. [Domain structure / List of seq. sharing at least 1 domain]
TIGRFAMs	TIGR00192; urease_beta; 1. TIGR00193; urease_gam; 1.
HOBACGEN	[Family / Alignment / Tree]
BLOCKS	Q9ZMZ4.
ProtoNet	Q9ZMZ4.
ProtoMap	Q9ZMZ4.
PRESAGE	Q9ZMZ4.
DIP	Q9ZMZ4.
ModBase	Q9ZMZ4.
SMR	Q9ZMZ4; A10B9DC4156C0561.
SWISS-2DPAGE	Get region on 2D PAGE.
UniRef	View cluster of proteins with at least 50% / 90% identity.

Keywords

Hydrolase; Complete proteome.

Features

None

Sequence information

Length: **238** Molecular weight: **26567** CRC64: **A10B9DC4156C0561** [This is a checksum on the

AA **Da** sequence]

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NEGKKAVSVK VKNVGDRPVQ IGSHFHFFEV NRCLDFDREK TFGKRLDIAS GTAVRFEPGE

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EKSVELIDIG GNRRIFGFNA LVDRQADNES KKIALHRAKE RGFHGAKSDD NYVKTIKE
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☐	L2	L1 and (modif$ or alter$ or mutant$ or mutation$ or mutagen$ or insert$ or heterologous$ or recombin$ or foreign$).clm.	24
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☐	L5	L3 same (modif$ or alter$ or mutant$ or mutation$ or mutagen$ or insert$ or heterologous$ or recombin$ or foreign$).clm.	3
☐	L6	l4 not l5	19
☐	L7	l4 not l5	19
☐	L8	salmonell$.clm.	865
☐	L9	L8 near5 (modif$ or alter$ or mutant$ or mutation$ or mutagen$ or insert$ or attenuat$ or avirulent$).clm.	60
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END OF SEARCH HISTORY

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Search Results - Record(s) 1 through 8 of 8 returned.

☐ 1. 6585975. 01 Nov 99; 01 Jul 03. Use of Salmonella vectors for vaccination against helicobacter infection. Kleanthous; Harold, et al. 424/200.1; 424/234.1 435/6 435/69.1 514/44 536/23.5. A61K039/02.

☐ 2. 6342215. 01 Dec 98; 29 Jan 02. Identification of genes. Holden; David William, et al. 424/93.2; 435/252.1 435/252.4 530/350 536/23.7. A61K048/00 C12N001/20 C12N015/31 C07K014/255.

☐ 3. 6190669. 13 May 98; 20 Feb 01. Attenuated mutants of salmonella which constitutively express the Vi antigen. Noriega; Fernando R., et al. 424/258.1; 424/831 424/93.1 424/93.2 424/93.4 424/93.48 435/879. A61K039/112.

☐ 4. 6030624. 15 Aug 97; 29 Feb 00. Mucosal immunogens for novel vaccines. Russell; Michael William, et al. 424/200.1; 424/244.1 424/93.2 435/252.3 435/252.8. A61K039/02 A61K039/09 C12N001/21.

☐ 5. 5877159. 03 May 95; 02 Mar 99. Method for introducing and expressing genes in animal cells and live invasive bacterial vectors for use in the same. Powell; Robert J., et al. 514/44; 424/184.1 424/93.1 424/93.21 424/93.4 435/235.1 435/320.1 435/472 435/480 435/69.1 536/24.1. A01N043/04 A61K031/70 C12N015/63.

☐ 6. 5733760. 05 Aug 94; 31 Mar 98. Salmonella vectors encoding truncated pag fusion protein, method of making, and uses thereof. Lu; Yichen, et al. 435/477; 424/185.1 424/190.1 424/192.1 424/200.1 424/208.1 424/234.1 424/258.1 435/245 435/252.3 435/320.1. C12N015/00 C12N015/63 A61K039/21 A61K039/112.

☐ 7. 5695983. 06 Jul 94; 09 Dec 97. Salmonella vaccines. Miller; Samuel I., et al. 435/252.8; 435/245. C12N001/20 C12N001/36.

☐ 8. 5424065. 19 Nov 92; 13 Jun 95. Vaccines containing avirulent phop-type microorganisms. Curtiss, III; Roy, et al. 424/93.2; 424/184.1 424/93.48 435/252.3 435/252.8 435/69.1 435/71.1. A61K039/02 C12N001/21.

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☐ 1. 6585975. 01 Nov 99; 01 Jul 03. Use of Salmonella vectors for vaccination against helicobacter infection. Kleanthous; Harold, et al. 424/200.1; 424/234.1 435/6 435/69.1 514/44 536/23.5. A61K039/02.

☐ 2. 6342215. 01 Dec 98; 29 Jan 02. Identification of genes. Holden; David William, et al. 424/93.2; 435/252.1 435/252.4 530/350 536/23.7. A61K048/00 C12N001/20 C12N015/31 C07K014/255.

☐ 3. 6190669. 13 May 98; 20 Feb 01. Attenuated mutants of salmonella which constitutively express the Vi antigen. Noriega; Fernando R., et al. 424/258.1; 424/831 424/93.1 424/93.2 424/93.4 424/93.48 435/879. A61K039/112.

☐ 4. 6030624. 15 Aug 97; 29 Feb 00. Mucosal immunogens for novel vaccines. Russell; Michael William, et al. 424/200.1; 424/244.1 424/93.2 435/252.3 435/252.8. A61K039/02 A61K039/09 C12N001/21.

☐ 5. 5877159. 03 May 95; 02 Mar 99. Method for introducing and expressing genes in animal cells and live invasive bacterial vectors for use in the same. Powell; Robert J., et al. 514/44; 424/184.1 424/93.1 424/93.21 424/93.4 435/235.1 435/320.1 435/472 435/480 435/69.1 536/24.1. A01N043/04 A61K031/70 C12N015/63.

☐ 6. 5733760. 05 Aug 94; 31 Mar 98. Salmonella vectors encoding truncated pag fusion protein, method of making, and uses thereof. Lu; Yichen, et al. 435/477; 424/185.1 424/190.1 424/192.1 424/200.1 424/208.1 424/234.1 424/258.1 435/245 435/252.3 435/320.1. C12N015/00 C12N015/63 A61K039/21 A61K039/112.

☐ 7. 5695983. 06 Jul 94; 09 Dec 97. Salmonella vaccines. Miller; Samuel I., et al. 435/252.8; 435/245. C12N001/20 C12N001/36.

☐ 8. 5424065. 19 Nov 92; 13 Jun 95. Vaccines containing avirulent phop-type microorganisms. Curtiss, III; Roy, et al. 424/93.2; 424/184.1 424/93.48 435/252.3 435/252.8 435/69.1 435/71.1. A61K039/02 C12N001/21.

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Terms	Documents
L9 and (pylori or pyloris or pyloridis or pylorum or pylor or hpylori or helicobacter or helicobact$)	8

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L10: Entry 1 of 8 File: USPT Jul 1, 2003

US-PAT-NO: 6585975
DOCUMENT-IDENTIFIER: US 6585975 B1

TITLE: Use of Salmonella vectors for vaccination against helicobacter infection

DATE-ISSUED: July 1, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Kleanthous; Harold	Westford	MA		
Londono-Arcila; Patricia	London			GB
Freeman; Donna	Cambridge			GB
Lee; Cynthia K.	Needham	MA		
Monath; Thomas P.	Harvard	MA		

US-CL-CURRENT: 424/200.1; 424/234.1, 435/6, 435/69.1, 514/44, 536/23.5

CLAIMS:

What is claimed is:

1. A method of inducing an immune response against Helicobacter in a mammal, said method comprising the steps of: mucosally administering to said mammal an attenuated Salmonella vector comprising a nucleic acid molecule encoding a Helicobacter antigen, and parenterally administering to said mammal a Helicobacter antigen.

2. The method of claim 1, wherein said attenuated Salmonella vector is administered orally to said mammal.

3. The method of claim 1, wherein said Helicobacter antigen is a urease, a urease subunit, or an immunogenic fragment thereof.

4. The method of claim 1, wherein said mammal is at risk of developing, but does not have, a Helicobacter infection.

5. The method of claim 1, wherein said mammal has a Helicobacter infection.

6. The method of claim 1, wherein said parenteral administration of said Helicobacter antigen further includes parenteral administration of an adjuvant.

7. The method of claim 6, wherein said adjuvant is an aluminum compound.

8. The method of claim 7, wherein said aluminum compound is alum.

9. The method of claim 1, wherein said attenuated Salmonella vector is a Salmonella typhi vector.

10. The method of claim 9, wherein said Salmonella typhi vector is CVD908-htrA or CVD908.

11. The method of claim 1, wherein the attenuated Salmonella vector is a Salmonella typhimurium vector.

12. The method of claim 11, wherein said Salmonella typhimurium vector is BRD509 or BRD807.

13. The method of claim 1, wherein said attenuated Salmonella vector further comprises an htrA promoter.

14. The method of claim 1, wherein said attenuated Salmonella vector further comprises a nirB promoter.

15. The method of claim 1, wherein said mucosal administration primes an immune response to an antigen and said parenteral administration boosts an immune response to said antigen.

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L10: Entry 1 of 8 File: USPT Jul 1, 2003

US-PAT-NO: 6585975
DOCUMENT-IDENTIFIER: US 6585975 B1

TITLE: Use of Salmonella vectors for vaccination against helicobacter infection

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Lee; Cynthia K.	Needham	MA		
Monath; Thomas P.	Harvard	MA		

US-CL-CURRENT: 424/200.1; 424/234.1, 435/6, 435/69.1, 514/44, 536/23.5

CLAIMS:

What is claimed is:

1. A method of inducing an immune response against Helicobacter in a mammal, said method comprising the steps of: mucosally administering to said mammal an attenuated Salmonella vector comprising a nucleic acid molecule encoding a Helicobacter antigen, and parenterally administering to said mammal a Helicobacter antigen.

2. The method of claim 1, wherein said attenuated Salmonella vector is administered orally to said mammal.

3. The method of claim 1, wherein said Helicobacter antigen is a urease, a urease subunit, or an immunogenic fragment thereof.

4. The method of claim 1, wherein said mammal is at risk of developing, but does not have, a Helicobacter infection.

5. The method of claim 1, wherein said mammal has a Helicobacter infection.

6. The method of claim 1, wherein said parenteral administration of said Helicobacter antigen further includes parenteral administration of an adjuvant.

7. The method of claim 6, wherein said adjuvant is an aluminum compound.

8. The method of claim 7, wherein said aluminum compound is alum.

9. The method of claim 1, wherein said attenuated Salmonella vector is a Salmonella typhi vector.

10. The method of claim 9, wherein said Salmonella typhi vector is CVD908-htrA or CVD908.

11. The method of claim 1, wherein the attenuated Salmonella vector is a Salmonella typhimurium vector.

12. The method of claim 11, wherein said Salmonella typhimurium vector is BRD509 or BRD807.

13. The method of claim 1, wherein said attenuated Salmonella vector further comprises an htrA promoter.

14. The method of claim 1, wherein said attenuated Salmonella vector further comprises a nirB promoter.

15. The method of claim 1, wherein said mucosal administration primes an immune response to an antigen and said parenteral administration boosts an immune response to said antigen.

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☐	L9	igg2a or igg-2a or (igg near2 2a)	3159
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☐	L12	(L11 or l10) same (l9 or l8)	3

END OF SEARCH HISTORY

08: Animal Models and Vaccines

8/1* Immunization-Induced Protection against *H. pylori* Is Impaired in Mast Cell-Deficient *Kit(W)/Kit(W-v)* Mice.

P. Michetti[1], M.Y. Wang[2], M. Michetti[1], B.K. Wershil[2]. [1]*Beth Israel Deaconess Medical Center, Harvard Medical School: Boston, MA United States;* [2]*SUNY Brooklyn University Health Center: Brooklyn, NY United States*

Background/Aim: The mechanisms by which oral immunization leads to protection against *H. pylori* are only partly elucidated. $CD4^+$ TH2 cells and alpha4beta7 integrin-mediated mucosal homing are required but their role in bacterial clearance remain unclear. Mucosal mast cells, which respond to TH2 stimulation and express alpha4beta7 integrin, have been implicated in the clearance of bacterial infections. The aim of this study was to determine whether mast cells are required for a protective immune response against *H. pylori*.

Methods: Mast cell-deficient *Kit(W)/Kit(W-v)* mice (mutant) and congenic +/+ (wt) were immunized 4 times at weekly intervals with *H. pylori* SS1 sonicates (Hp) + cholera toxin (CT) or CT only, and challenged 2 weeks later with 10^8 *H. pylori* SS1. Protection (defined as negative rapid urease test and negative histology) was evaluated 5 weeks after challenge. Specific serum IgG and IgA titers were determined by ELISA.

Results: Following immunization with Hp+CT, 14/17 wt mice (82%) and 2/17 mutant mice (12%) were protected against *H. pylori* SS1 ($p < 0.0001$; Fisher Exact). No protection was observed in wt or mutant CT-immunized mice. Among Hp+CT immunized animals, median IgG and IgA titers were $1{:}8x10^5$ and $1{:}16x10^3$, resp. in mutant mice and $1{:}4x10^5$ and $1{:}4x10^3$, resp. in wt mice (endpoint dilutions; p=NS).

Conclusions: Protection against *H. pylori* is markedly diminished in mast cell-deficient *Kit(W)/Kit(W-v)* mice despite functional TH2 and B cell responses. These results suggest that mast cells, or other defects secondary to *W* mutations participate in the immunity to *H. pylori*. NIH DK53706.

8/2* Inflammatory Bowel Disease with Increased IL-12 Expression in NF-kB Deficient Mice.

S.E. Erdman[1], J.G. Fox[1], C.A. Dangler[1], B.H. Horwitz[2]. [1]*Massachusetts Institute of Technology: Cambridge, MA United States;* [2]*Brigham and Women's Hospital: Boston, MA United States*

Severe inflammatory bowel disease (IBD) was observed in *Helicobacter hepaticus*-infected mice lacking genes for nuclear factor kB (NF-kB). In this study, p50- and p65-deficient mice on a mixed 129 x C57BL/6 were rederived by uterine embryo transfer and maintained as specific pathogen free for known enterohepatic Helicobacter species. At age 6–8 weeks, mice of each genotype were infected with *H. hepaticus* and controls received media only. At six weeks postinfection, typhlocolitis was significantly more severe in *H. hepaticus*-infected p65+/–, p50-/- mice and p65+/+, p50-/- mice than in the infected wild type controls or uninfected controls of all genotypes. The inflammation of the cecum and ascending and transverse colon was characterized by marked hyperplasia of the mucosal crypts, multifocal mucosal erosion and ulceration, and effacing mononuclear inflammatory infiltrates consisting primarily of macrophages and T lymphocytes. Upregulation of proinflammatory cytokines was suggested by increased IL-12 expression in cecal and colonic biopsies compared with controls using RNAse protection assays. Increases in INF-gamma suggested a pathogenic Th1 response and increases in IL-1alpha and IL-6 indicated involvement of lamina propria macrophages. The initial observation of IBD in NF-kB deficient mice was unexpected because NF-kB transcription factors have been implicated as promoters of IBD. This data suggests that p50 and p65 may have a much broader role in inhibition of inflammatory gene expression than previously appreciated.

8/3 Oral Immunization against *H. pylori* in Healthy Volunteers with Low Dose *E. coli* Enterotoxin (LT) and Recombinant Urease.

S. Banerjee[1], A. Medina-Fatimi[1], R. Nichols[2], D. Tendler[1], M. Michetti[1], M. Mach[1], J. Simon[2], C.P. Kelly[1], T.P. Monath[2], **P. Michetti**[1]. [1]*Beth Israel Deaconess Medical Center, Harvard Medical School: Boston, MA United States;* [2]*OraVax Inc.: Cambridge, MA United States*

Backgroud: Limited evidence suggests LT can be used as a adjuvant in oral vaccines against *H. pylori*, but the optimal dose of LT is not defined. This study was undertaken to find the lowest effective dose of LT and to evaluate the safety and immunogenicity of *H. pylori* urease delivered in enteric-release capsules.

Methods: 42 healthy adults without *H. pylori* infection were randomized to 60mg urease in soluble or encapsulated form, given 4 times with LT doses ranging from 0 to 2.5ug. Circulating urease specific antibody-secreting cells (ASC) were measured by ELISPOT and serum antibody responses by ELISA. Response was defined as a 4-fold increase in serum IgA or IgG titers or by >15 ASC/10^6 cells. Circulating lymphocytes expressing alpha4beta7 integrin (mucosal homing receptor) and CD4, CD19, CD45, or CD69 were enumerated.

Results:: Mild diarrhea (1–4 loose stools) occurred in 6/12 volunteers (50%) exposed to 2.5ug LT (p=0.06; paired *t*-test vs. baseline) but not in volunteers exposed to lower LT doses or to urease alone. Proportions of responders were 67%, 17%, 33%, and 17% among subjects exposed to 2.5ug (p=0.048; Fisher Exact vs. no LT), 0.5ug, 0.1ug and no LT, respectively. alpha4beta7$^+$ lymphocytes co-expressing CD4, CD69, and CD45 increased only in volunteers exposed to 2.5ug LT. Urease in enteric-release capsules appeared more immunogenic than soluble urease (n=3 per group).

Conclusions: Oral immunization with recombinant *H. pylori* urease and a low dose of LT is safe and can induce humoral and cellular mucosal immune responses in healthy volunteers.

8/4* Protection Against Murine *Helicobacter pylori* Infection after Urease Immunization is Dependent on IFNgamma and IL-12 and is Regulated by IL-10.

T.H. Ermak[1], T. Tibbitts[1], H. Gray[1], W. Lei[1], R. Nichols[1], G.A. Myers[1], P.J. Giannasca[1], J. Hill[2], T.P. Monath[1], H. Kleanthous[1]. [1]*OraVax, Inc.: Cambridge, MA United States;* [2]*Clemson Univ.: Columbia, SC United States*

Protection against experimental *H. pylori* infection in mice after immunization with urease is dependent on MHC class II-restricted cell mediated immunity.

We used knockout (KO) mice in two studies to examine 1) the roles of IL-12 (a Th1 cytokine) and IL-10 (a T-regulatory cytokine) and 2) the roles of IFNgamma (a Th1 cytokine) and IL-4 (a Th2 cytokine) in protection. C57BL/6J wildtype (WT) and KO mice were immunized either by the rectal route with urease plus *Escherichia coli* heat-labile toxin (LT) or by subcutaneous injection with urease plus alum. Control mice received no immunization. Two weeks after the last immunization, all groups of mice were challenged with *H. pylori* strain X47-2AL and protection assessed 2 weeks later.

In WT mice, mucosal immunization decreased *H. pylori* CFUs by over 2 $\log_{10}$, and parenteral immunization decreased CFUs by 0.5 $\log_{10}$ relative to unimmunized WT controls. In the absence of IL-12 or IFNgamma, KO mice showed a reduction in protective efficacy: IL-12 and IFNgamma KO mice were not protected when immunized by the parenteral route, and had a 1-$\log_{10}$ reduction in bacterial burden when immunized by the mucosal route. In contrast, IL-10 KO but not IL-4 KO mice showed greater protection than did WT mice, with a 3- $\log_{10}$ decrease in CFUs after mucosal immunization and a 1-$\log_{10}$ decrease after parenteral immunization relative to unimmunized controls.

These results indicate that protection against *H. pylori* in the mouse is effected by an IFNgamma- and IL-12-dependent, IL-10-regulated, Th1 response.

8/5* Resistance to *Helicobacter pylori* Infection is Associated with Severe Gastritis in B Cell Deficient Mice.

A.A. Akhiani[1], K. Schön[1], L.E. Franzen[2], J. Pappo[3], N. Lycke[1]. *[1]University of Göteborg: Göteborg, Sweden; [2]Örebro University Hospital: Örebro, Sweden; [3]AstraZeneca R&D Boston: Boston, MA United States*

Recent studies in B cell deficient (muMT) mice have suggested that *H. pylori* specific antibodies play a subordinate role in protection against *H. pylori* infection. Here we have asked whether lack of antibodies would influence the susceptibility to a primary infection with *H. pylori* and to what degree gastric immunopathology and bacterial colonization are interrelated. We found that 8 week-infected muMT mice exhibited at least a 100-fold reduction in bacterial load, which was associated with a significantly more severe gastric inflammation compared to that seen in wild type (WT) mice. Interestingly, and contrary to WT mice, eosinophilic leukocytes dominated the inflammatory cells together with CD4+ T cells. Thus, muMT mice exhibited a severe gastritis but reduced bacterial load suggesting an inverse correlation between the bacterial load and the severity of gastritis. A similar pattern of immunopathology was also observed in both muMT and WT mice orally immunized with *H. pylori* lysate and cholera toxin (CT) after challenge with live bacteria. The gastric inflammation was as severe in the immunized/challenged mice as in the primary infected muMT mice. Furthermore, T cells from protected muMT as well as WT mice produced high levels of IFN-gamma but undetectable levels of IL-4 and IL-5, upon stimulation with recall antigen in vitro. These findings in the muMT mice suggest that IFN-gamma and Th1 cells appear to play a significant role for protection against *H. pylori* infection despite being critical for the development of gastritis.

8/6 Identification of Potential Vaccine Candidates from *Helicobacter pylori* Using Proteomics Technologies.

M.J. Fiske, J. Caplan, M. Wetherell, J. Fulginiti, D. Chakravarti. *Wyeth-Lederle Vaccines: West Henrietta, NY United States*

Objective: For the gastric pathogen *H. pylori* we have applied proteomics technologies for the identification of a series of monoclonal antibody reactive, cell surface exposed protein antigens from N-terminal sequence tags. In separate studies we have utilized proteomics tools for the rapid analysis of an outer membrane protein (OMP) fraction using 2-D gel electrophoresis and mass spectrometry.

Methods: SDS-PAGE in combination with Western blot and N-terminal sequence analysis were used to separate and identify protein antigens reactive with a series of monoclonal antibodies raised against intact *H. pylori* cells. Following trypsin digestion of protein spots separated by 2-D PAGE, MALDI-TOF mass spectrometry was carried out for the direct identification of the protein antigens.

Results: We have identified over 25 monoclonal antibody reactive proteins from SDS-PAGE, N-terminal sequence analysis, and sequence database searching. Using 2-D PAGE, trypsin digestion and MALDI-TOF mass spectrometry, a number of individual proteins were identified in the OMP preparation by comparison of the peptide mass fingerprint to existing sequence databases. Several of these candidates identified were shown to reduce colonization of *H. pylori* strain SS1 in our prophylactic mouse model.

Discussion: The use of proteomics has proven useful for the rapid identification of surface exposed and membrane associated *H. pylori* proteins. This approach should speed up the identification of potential vaccine protein antigens for microbial pathogens in general.

8/7 Treatment of *H. pylori* Infected BALB/c Mice with a Bovine Immunoglobulin Concentrate Containing High Amounts of Anti-*H. pylori* IgG-Antibodies.

T.H. Casswall[1], H.O. Nilsson[2], T. Borén[3], T. Wadström[2], L. Hammarström[4]. *[1]Clinical Science: Huddinge, Sweden; [2]Medical Microbiology: Lund, Sweden; [3]Oral Biology: Umeå, Sweden; [4]Clinical Immunology: Huddinge, Sweden*

Objective: The emerging development of antibiotic resistance comprises a need for new treatment strategies of *H. pylori* infection. Animal studies have suggested a therapeutic role of increased gastric antibody levels against *H. pylori*, achieved either by active or passive oral immunisation. Hence, we used a bovine immunoglobulin concentrate (BIC) in two treatment trials in a *H. pylori* mouse model.

Methods: The lypohilised BIC preparation was produced from colostrum from Swedish cows immunised 8 times with a mixture of formalin killed *H. pylori* CCUG 17874, 17875 and a local clinical isolate (strain 33). The BIC preparation contained 84.1% protein out of which 54.6% was bovine IgG. The product contained 100 times more specific anti-*H. pylori* antibodies and 40 times more specific anti-urease antibodies than colostrum from non-immunised cows. The antibody preparations were capable of blocking the adherence of *H. pylori* to gastric mucosa in vitro and to the Lewis^b antigen by its receptor, BabA. BALB/c mice were orally twice infected by gavage with 5 x 10^8 bacteria of a *H. pylori* strain 119–97, in two different trials. After 11–15 days of incubation treatment was started for 10 (trial 1) or 20 days (trial 2) with either BIC 10, 20 or 50 mg/mL or control (BIC in low concentration, 0.1 mg/mL), non-immunised colostrum from animals or no treatment, i.e., water) dissolved in the drinking water. After treatment the mice were sacrificed and gastric mucosal samples (1/3 of the mouse stomach) were cultured for *H. pylori*.

Results: In the 1st trial, 2 of 20 mice vs. 17 of 38 in the control group ($p < 0.01$) were still infected and in the 2nd trial, 5 of 20 mice (BIC 20 mg/mL) vs 8 of 10 control mice (water) ($p < 0.01$) were still infected. Adding both trials together 7 of 40 BIC treated mice vs. 24 of 48 the control mice were still infected ($p < 0.001$). The overall cure rate was 65%. The mean numbers of colonies in the mice, which were still infected in both trials, were also reduced by 64% in the BIC treated group as compared to the infected mice in the different control groups (18 vs. 50, $p < 0.01$).

Discussion: Taking the nature of the crude antibody preparation into account a cure rate of 65% is surprisingly high. Furthermore, the bacterial load was reduced by 64% of the BIC treated mice who were still infected, which further supports the feasibility of this approach. However, the pathophysiological properties differ between mice and humans and results in animals can only serve as an indication of a beneficial potential.

8/8 Inhibition of *H. pylori* Infection by Bovine Milk Glycoconjugates in a Mouse Model.

X. Wang[1], S. Hirmo[1], R. Willén[2], T. Wadström[1]. *[1]Lund University: Lund, Sweden; [2]Sahlgrenska University Hospital: Gothenburg, Sweden*

The attachment of *H. pylori* to the human gastric mucosa is a complex process involving several specific structures recognised by the cell surface receptors. Sialylated multivalent high molecular weight glycoproteins have been shown to inhibit *H. pylori* sialic acid specified haemaggultination. This study was aimed to explore whether sialylated glycoconjugates from bovine milk can inhibit an experimental *H. pylori* infection in a Balb/cA mouse model.

Sixty 6–8 weeks old Balb/cA mice were used in this study. Fifty mice were inoculated by *H. pylori* a mouse-passaged strain 317p and divided into five groups (n=10 per group). Four groups of mice were given lactoferrin (iron-free or 20% iron-saturated) or bovine milk fat globule membrane fractions (MFGM or defatted MFGM) orally 400 mg/kg once daily for 10 days at 4-week post-inoculation. Ten uninfected mice and ten infected control mice were given water. All mice were sacrificed immediately after the treatment, stomach culture and histopathology were performed.

The infected control mice were all culture positives for *H. pylori*. The mice treated with lactoferrin iron-free, lactoferrin 20% iron-saturated, MFGM or defatted-MFGM showed 30%, 10%, 20% or 20% of recovery rate compared to non-treated control, respectively. The gastric colonisation of *H. pylori* was remarkably decreased in all bovine milk glycoconjugates treated mice. The inflammation score of the stomachs was significantly lower in treated mice than in infected control animals, but still higher than in uninfected controls.

In conclusion, bovine milk glycoconjugates inhibit *H. pylori* infection in this mouse model and should be considered as candidates for a non-antibiotic strategy to combat *H. pylori* infections in humans.

8/9 Protective effect of cross-reactive region$_{189-203rd}$ of *Helicobacter pylori* heat shock protein 60 (HSP60) on *H. pylori* infection.

H. Yamaguchi, A. Toyoda, M. Takahashi, H. Taguchi, S. Kamiya. *Kyorin University School of Medicine: Mitaka, Japan*

Title: Protective effect of cross-reactive region $_{189-203rd}$ of *Helicobacter pylori* heat shock protein 60 (HSP60) on *H. pylori* infection.

Objectives: To evaluate the efficacy of cross-reactive region $_{189-203rd}$ of *Helicobacter pylori* HSP60 as avaccine, the protective effect of immunizing mice with the region was investigated.

Methods: The amino acid sequences corresponding to residues $_{189-203rd}$ and $_{463-477th}$ of the *H. pylori* HSP60, which were designated pH9 and pCont (control peptide), respectively, were synthesized. SPF C57BL/6 mice were i.p. immunized five times on a weekly schedule with either pH9 peptide plus FCA (n=11), pCont peptide plus FCA (n=10), or FCA only (n=10). Nonimmunized mice without *H. pylori* infection were used as controls in ELISA analysis (n=7). One week after the last immunization, the mice were orally infected three times with *H. pylori* clinical isolate TK1402 (*vacA* +, *cagA*+). Two weeks after infection, the mice were sacrified. Serum was obtained from each mouse for ELISA, and the number of bacteria colonizing the stomach was determined.

Results: The number of *H. pylori* colonizing the stomach mucosa of mice immunized with pH9 plus FCA was significantly lower than that in mice immunized with either pCont plus FCA or FCA only (259+/−65 vs 4939+/−827 [$p < 0.0001$] or vs 6663+/−2068 [$p < 0.005$]). Values of IgG against pH9 in the mice immunized with pH9 plus FCA were significantly higher than those in mice immunized with pCont plus FCA, FCA only or control mice (0.479+/−0.042 vs 0.088+/−0.006 [$p < 0.0001$], vs 0.079+/−0.015 [$p < 0.0001$], or vs 0.036+/−0.014 [$p < 0.0001$]). In contrast, no significant IgG response against PPD was observed in any mice groups.

Conclusion: The results suggest that the immune response to the cross-reactive region (pH9) on *H. pylori* HSP60 is associated with protection against *H. pylori* infection.

8/10 Short Term Infection with Helicobacter pylori and Exposure to Metronidazole does not enhance the Gastric Mutation Frequency in Big Blue® Transgenic Mice.

E. Touati[1], M. Hofnung[1], J. Thiberge[1], V. Michel[1], A. Labigne[1], **P.J. Jenks[2]**. *[1]Institut Pasteur: Paris, France; [2]Royal Free Hospital Medical School: London, United Kingdom*

Objective: The aim of this study was to determine whether exposure of *Helicobacter pylori*-infected mice to metronidazole resulted in the delivery of mutagenic compounds to the gastric epithelium via the oxygen-insensitive NADPH nitroreductase (RdxA) of H. pylori.

Methods: C57BL/6 mice containing the lambda/lacI transgene (Big Blue transgenic mice) were inoculated with peptone trypsin broth, *H. pylori* SS1, or SS1-rdxA- , an SS-derived mutant in rdxA. Twelve weeks after inoculation, the mice were treated for 7 days with either a control solution or the mouse equivalent of 1 g metronidazole once a day. Three weeks after the completion of treatment, the animals were sacrificed and mutations in the target lacI gene assessed using the Big Blue transgenic mutagenesis assay system.

Results: There was no increase in lacI mutations in cells harvested from mice infected with *H. pylori* and/or exposed to metronidazole.

Discussion: This data suggests that short term infection with *Helicobacter pylori* and exposure to metronidazole does not enhance the mutation frequency in the gastric cells of mice. Whether chronic infection and/or repeated exposure to metronidazole or other nitroaromatic compounds causes genetic damage to gastric epithelial cells remains to be determined.

8/11 Amelioration of Gastric Inflammation by Polyphenols from Red Wine: A Mouse Model using *Helicobacter felis*.

K. Mayo, C. Castagnino, C. Chèze, J. Vercauteren, A. de Mascarel, F. Mégraud. *University of Bordeaux 2: Bordeaux, France*

Aim: to test the effect of total polyphenolic content of red wine on inflammation caused by chronic infection of the stomach which persists long after the infection is cured, using the model of *Helicobacter felis* infection in C56/BL6 mice.

Methods: Five groups of 10 mice were orally inoculated with *H. felis* and a sixth group with sterile Brucella broth. One month later, two groups of inoculated mice received eradication treatment (clarithromycin, 1 mg, amoxicillin, 1 mg, and omeprazole, 0.02 mg, once daily for 1 wk). Then, one group which had received eradication treatment and one group which had not were given polyphenols extracted from red wine (approximately 500 mg/day/mouse) plus 10% ethanol in drinking water. Two control groups corresponding to these two treatment groups received 10% ethanol only. Another group of infected mice not receiving eradication treatment was given unadulterated drinking water. After 2 weeks, stomachs were removed, and prepared for histological examination and graded using the Sydney system. Mean scores of histological parameters were interpreted using analysis of variance.

Results: There was a significant difference in the mean score of lymphocyte infiltration between the group treated with polyphenols after eradication (0.7) and all other groups except the healthy control (0.3) ($p < 0.05$). There was no significant difference in this parameter among the four other groups: eradicated/placebo (1.3), non-eradicated/polyphenols (1.7), non-eradicated/placebo (1.7), non-eradicated/placebo (no ethanol) (1.5). Polyphenol ingestion after eradication treatment significantly reduced lymphocyte infiltration but not polymorphonuclear leukocyte infiltration. Lymphoid follicles were observed in all groups except the group receiving polyphenols after eradication and the healthy controls. The effects of polyphenol treatment were observed only after eradication of the bacteria.

Conclusion: The slow healing of the gastric mucosa after *H. felis* eradication was significantly improved by comsumption of polyphenols. Possible mechanisms include the previously demonstrated anti-oxidant properties of these compounds.

8/12 Mucosal Immunization by Hsp60 Induced Early Gastritis in *H. pylori* Infected Mice.

K. Yokota[1], E. Ishii[1], K. Ayada[1], T. Toyokawa[1], K. Kobayashi[2], Y. Kawahara[3], S. Hayashi[4], Y. Hirai[4], M. MIzuno[1], T. Tsuji[1], K. Oguma[1]. *[1]Okayama University Medical School: Okayama, Japan; [2]Fukuyama National Hospital: Okayama, Japan; [3]Tsuyama Central Hospital: Tsuyama, Japan; [4]Jichi Medical School: Yakushiji, Japan*

Objective: Heat shock protein (hsp) 60 is an immune dominant antigen in *H. pylori* infection. This study aimed to evaluate immunological role of hsp60 by a rodent infectious model.

Methods: Whole hsp60 and two partial regions of hericobacterial hsp60 were expressed by GST-fusion proteins, and were designated rHSPw, rHSP2, and rHSP4–5. rHSPw was expressed as whole hsp60 (Met_1-Met_{545}). rHSP2 (Glu_{101}-Ser_{200}) contained a domain of T cell epitope cluster (Lys_{159}-Thr_{178}). rHSP4–5 (Ile_{300}-Gly_{435}) contained a domain of T cell epitope cluster (Asp_{396}-Gly_{412}) and its upstream of region (Ser_{356}-Asp_{392}) common to *H. pylori* and human hsp60. Recombinant heat-lebile enterotoxin (rLT) of *E. coli* was also employed as adjuvant for nosal immunization. Three different mouse groups (BALB/c, C3H/He, C57BL/6) were injected with 10^8 CFU of live *H. pylori* (SS -1) twice a week and kept in a clean isolator for 2 weeks. Those mice were immunized *H. pylori* lysate or rHSPs (10μg/mouse) mixed with rLT (10μg/mouse) by injecting into nosal cavity four times in 1 month. Mice were sacrificed at 1 month after the last immunization, and then histopathology and level of antibodies were investigated.

Results: Mucosal immunization by *H. pylori* lysate and rHSPs induced severe gastritis in BALB/c and C57BL/6 mice. Especially, the inflammations were very severe in those two mice groups immunized by rHSP4–5. Mucosal immunization by hsp60 accelerated the production of IgG and IgA against *H. pylori*.

Discussion: Results indicated that hsp 60 was closely associated with *H. pylori* induced gastric inflammation.

8/13 IgG1-Mediated Immune Exclusion of *H. pylori* in vivo.

J. Keenan, R. Allardyce. *Christchurch School of Medicine: Christchurch, New Zealand*

Specific humoral and cell-mediated immunity both contribute to protective immunity against *H. pylori*. However, the mechanisms of protection are poorly understood.

Objectives: To demonstrate that IgG1-mediated immune exclusion contributes to clearance of *H. pylori* infection in a murine model.

Methods: *In vivo*: Backpack tumours were generated in *H. pylori* SS1-infected mice (n=20) by injecting IgG1 secreting hybridoma cells: MAb 6A8, specific for *H. pylori* Lpp20 = test and a non-specific sham antibody = control.

In vitro: *H. pylori* SS1 were incubated with heat-inactivated sera from these mice. Immunogold electron microscopy demonstrated the concentration-dependent anti-bacterial effects of specific antibody.

Results: At day 20, each animal carried a large backpack tumour. ELISA showed circulating MAb in all mice. This antibody was also detected in gastric lavages. Quantitative culture of gastric biopsies revealed fewer *H. pylori* CFUs in the mice with 6A8 backpacks ($P = 0.0002$). Gastric

histology of test mice showed increased aggregation of the remaining *H. pylori* when compared to controls. In vitro immunolabeling of *H. pylori* SS1 supported this observation.

Discussion: Reduced colonisation suggests specific IgG1 antibodies that leak from circulation into the stomach inhibit *H. pylori* persistence on the gastric epithelium. Our in vitro studies suggest that *H. pylori* mobility is impaired once a threshold level of antibody is reached. In consequence, immune exclusion by the host may over-ride immune evasion by the bacterium.

8/14 Variations of *H. pylori* Clinical Isolates during Murine Infection.

B. Janvier, S. Ayraud, L. Salaün, J.L. Fauchère. *CHU: Poitiers, France*

Objective. Clinical *H. pylori* isolates show a high diversity which could be due to adaptative changes of the strains passing from an host to another. In order to study those variations, mice were experimentally infected and infecting strains were compared to emerging strains during a one year follow up.

Methods. C57BL/6 mice were orally infected with 3 *H. pylori* infecting strains: 2 clinical isolates and the SS1 strain known to be adapted to the murine stomach. Animals were sacrified at days 3, 7, 14, 21, 45, 90, 120, 150, 240 and 360 post-infection. The emerging strains were cultured once and were compared to the infecting strains for proteic profile by SDS-PAGE, for antigenic profile by western blot, and for genomic variations by RAPD, PCR and MLEE (multilocus enzyme electrophoresis).

Results. No genotypic nor phenotypic variations were observed with the SS1 strain. For the 2 *H. pylori* clinical isolates, no genotypic variation were found while proteic profiles showed variations as soon as day 3. These variations consisted in an over-expression of a 180 kda protein and a decreased expression of proteins of 120 and 260 kDa, and persisted over the one year experimental infection. Moreover, antigenic analysis using monospecific sera showed modifications in the expression of CagA and VacA.

Conclusion. During murine infection, the SS1 which is well adapted to mice did not show any variation while the strains passing from a human to a murine host showed phenotypic changes. No genomic variations were detected.

8/15 Antibiotic-Associated *Clostridium difficile* Colitis in Mongolian Gerbils (*Meriones unguiculatus*) Treated with Amoxicillin/Metronidazole/Bismuth Wafers.

I.L. Bergin, N.S. Taylor, J.G. Fox. *Massachusetts Institute of Technology: Cambridge, MA United States*

The Mongolian gerbil (*Meriones unguiculatus*) has become an important model for the study of *Helicobacter pylori*-associated gastritis, peptic ulcer disease, and gastric adenocarcinoma. We have recently found that some commercially-available gerbils harbor naturally-occurring *H. bilis* infections. We utilized an existing protocol for the elimination of *H. hepaticus* infection in mice to attempt eradication of *H. bilis* in gerbils. This protocol involved the use of commercially available, nutritionally-balanced triple therapy wafers containing amoxicillin (3 mg/tablet), metronidazole (0.09 mg/tablet), and bismuth (0.185 mg/tablet) administered as a sole food source for fourteen days. For a preliminary study, five male Mongolian gerbils were fed the wafers. On day seven of treatment, two of the five animals were found dead. Necropsy and histopathology revealed a distended cecum and colon and moderate typhlitis and colitis suggestive of antibiotic-associated *C. difficile* colitis. The diagnosis was confirmed by positive ELISA for *C. difficile* toxins A and B performed on colonic tissue from the two animals. Additionally, anaerobic culture of cecal contents resulted in growth of *C. difficile* from one of the animals. Antibiotic-associated colitis, while common in hamsters, has not, to our knowledge, been reported in the gerbil.

Susceptibility of gerbils may vary depending on the animal source and the route or dose of antibiotics administered. Successful elimination of naturally-occurring Helicobacter infections in gerbils may require the identification of a *C. difficile*-free source for gerbils or alternate techniques such as Caesarian rederivation and cross-fostering onto mice.

This study was supported by NIH grant R01-A37750.

8/16 Mice are not Men: The Sydney System does not Discriminate the Genetically Determined Degree of Gastritis in *H. felis* Infection.

M.M. Walker, M.L. Worku, Q.N. Karim, M.R. Thursz. *ICSM at St Mary's: London, United Kingdom*

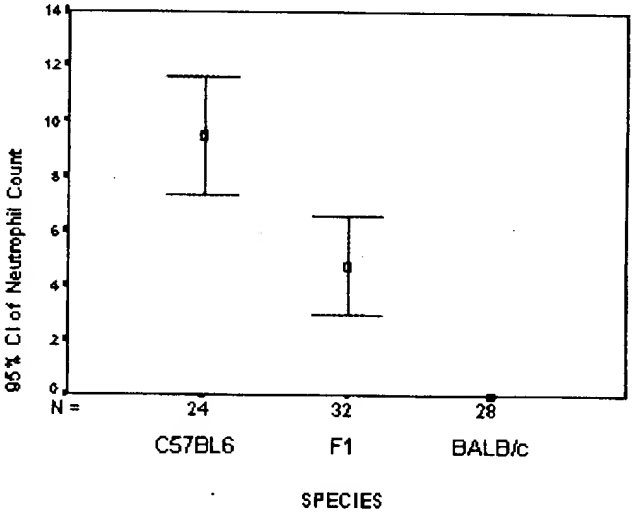
Objective: In the mouse model of Helicobacter infection it has been demonstrated that the degree of gastric mucosal inflammation is determined by mouse strain. This model is therefore a valuable tool for investigating the genetic determinants for the outcome of human Helicobacter infection. We aimed to devise a robust method for quantifying active inflammation to be used in identifying quantitative trait loci.

Methods: In a pilot study C57BL/6, BALB/c and (C57BL/6 x BALB/c) F1 mice were given either 10^8 *H. pylori* (Sydney strain) or 10^8 *H. felis* at 6 weeks of age by gavage on days 1, 3 and 5 of the experiment. The mice were sacrificed by humane terminal anaesthesia at 12 weeks. Whole mount sections of the lesser and greater curve of stomach were formalin fixed, routinely processed, stained with H&E and Giemsa to assess gastritis, atrophy and bacterial colonisation by site.

Results: Semiquantitative scoring for active inflammation, based on the Sydney system, was found to be inadequate to discriminate quantifiable differences in gastric inflammation between strains. Therefore we used a method which quantified the level of active inflammation, counting the number of neutrophils in inflamed gastric pits and taking the total number of neutrophils in 3 inflamed pits within each region (cardia, body transitional zone and antrum).

The degree of inflammation and colonisation observed with the *H. pylori* Sydney strain was exceedingly low and although there were phenotypic differences between the strains it was not sufficiently discriminatory to be useful. However, when infected with the *H. felis* strain there were clear differences between the two parental strains for the degree of active gastritis. The phenotype of the (C57BL/6 x BALB/c) F1 lay between the two parental extremes (see figure). C57BL6 v F1: $P = 0.003$, BALB/c v F1: $P < 0.001$, C57BL6 v BALB/c: $P < 0.0001$. (Mann Witney U test)

Conclusions: This method provides a numerical value for inflammation that is accurate (corresponds to the total neutrophil count per section), reproducible (not statistically different from the neutrophil count in separate sections taken from the same mouse) and discriminatory.



8/17 Identification of *Helicobacter mustelae* Virulence Factors by Screening of a Random Insertional Mutant Library.

T. O Croinin[1], S. Brands Heerma[2], B. Drumm[1], C.M.J. Vandenbroucke-Grauls[2], B. Bourke[1], J.G. Kusters[2]. [1]*University College Dublin: Dublin, Ireland;* [2]*Vrije Universiteit: Amsterdam, Netherlands*

Background: *Helicobacter mustelae* infection of ferrets has been associated with gastritis, duodenal ulcer disease and gastric cancer. Although experimental *Helicobacter pylori* infections have been developed, natural infection has only been described in humans and non-human primates. Therefore infection of ferrets with *H. mustelae* is recognised as an important natural animal model of *H. pylori* infection in humans. The aim

of this study was to develop a method for generating insertion mutants in *H. mustelae* with a view to identifying genes important in a natural Helicobacter infection.

Methods: *H. mustelae* chromosomal DNA was digested with ClaI and self ligated to create circular DNA. This DNA was then re-digested with Bgl II and recircularized by ligation with the aphA-3 kanamycin resistance cassette. The resultant aphA-3 containing circles were then naturally transformed into *H. mustelae* strain NCTC 12032. Successful introduction gives rise to homologous cross-over with corresponding genes in the chromosome resulting in disruption of a gene and conferring kanamycin resistance. Mutants were selected on blood agar plates containing kanamycin.

Results: Natural transformation of *H. mustelae* with the constructs resulted in 500 kanamycin resistant transformants per mg DNA. This indicates that *H. mustelae* is naturally competent for transformation with DNA. A Southern blot with 12 randomly selected transformants probed with the kanamycin cassette showed that the constructs integrated into different sites in the *H. mustelae* chromosome suggesting random integration. An initial mutant library of 500 mutants was created and screened for the absence of virulence factors. Screening for urease activity using Christensens broth has revealed two urease deficient mutants.

Conclusion: In this study we describe a method for generating a mutant library in *H. mustelae*. The ability to screen for *H. mustelae* mutants attenuated in virulence both in vitro and in vivo represents an important opportunity to investigate the pathogenesis of gastric Helicobacter species in their natural hosts.

8/18 Evaluation of *Helicobacter pylori* Stool Antigen Test (HpSA) for Detection of Infection in Mongolian Gerbils.

M. Court, P.A. Robinson, J.E. Crabtree. *St James's University Hospital: Leeds, United Kingdom*

Objective: A non-invasive test for determining *H. pylori* infection in laboratory rodents would be a useful adjunct for assessing infectivity of inoculated strains and monitoring colonisation temporally. An immunoassay for detecting *H. pylori* antigen in stools (HpSA) is diagnostically accurate for determining *H. pylori* infection in humans. The aim of this study was to evaluate if the assay was suitable for assessing infection in Mongolian gerbils.

Method: Male Mongolian gerbils aged 6–8 weeks were orally inoculated three times with 8 different *H. pylori* strains. At 6 weeks post-infection gerbils were sacrificed and gastric *H. pylori* infection assessed by biopsy urease test, culture and histology. Fresh stools were collected prior to sacrifice and analysed using the HpSA immunoassay (Meridian, Diagnostics Inc.). Faecal pellets (n=6) were dispersed in 500µl sample diluent and 50µl used for assay.

Results: The mean±SEM OD (450nm) in the HpSA assay was 0.065± 0.005 (n=21) in gerbils which were *H. pylori* negative by both culture and urease test. All samples were below the recommend negative value of 0.140 OD. The mean±SEM OD, 0.206±0.009 (n=5) in gerbils infected with *H. pylori* was significantly greater than in uninfected animals ($p < 0.0001$). All infected animals had an OD greater than 0.160, the recommended cut-off for positivity. A strong linear association was observed between stool weight and OD in infected animals, with weights greater than 0.1g giving positive titres in the HpSA assay.

Conclusion: The HpSA assay is useful for non-invasive detection of *H. pylori* in Mongolian gerbils allowing confirmation of infection, temporal changes in natural infection and the effects of pharmacological agents to be monitored.

8/19 Surface Ring-Mutant Strains of *H. mustelae* Fail to Persistently Colonize the Ferret Stomach.

M.M. Patterson[1], S. Xu[1], A. King[1], N.S. Taylor[1], R.P. Marini[1], N. Forester[2], P.W. O'Toole[2], B. Noonan[3], T.J. Trust[3], J.G. Fox[1]. *[1]Massachusetts Institute of Technology: Cambridge, MA United States; [2]Massey University: Palmerston North, New Zealand; [3]AstraZeneca R&D Boston: Waltham, MA United States*

Helicobacter mustelae has been linked to chronic gastritis, peptic ulcers and gastric cancer in ferrets. In contrast to *H. pylori*, the outer surface of *H. mustelae* is covered by an extensive array of 8.5 nm rings made of a protein designated Hsr. These surface rings are potentially analogous to the classical S-layer found ubiquitously among prokaryotes; functions ascribed to S-layers include roles in protection, cell adhesion, surface recognition and virulence. To examine the importance of Hsr in the pathogenesis of *H. mustelae*, two isogenic Hsr mutant strains were constructed by disrupting the hsr gene. Ferrets determined to be specific-pathogen-free for *H. mustelae* were dosed with 3×10^8 CFU of Hsr mutant 1 (three ferrets), Hsr mutant 2 (four ferrets), wild-type *H. mustelae* (one ferret), or sterile culture broth (two ferrets). Gastric biopsies for quantitative cultures were obtained at 3, 6, 9 and 12 weeks post-infection, with complete necropsies at 12 weeks. The sham-treated ferrets remained culture-negative at all timepoints. Using a one-sample t test, antral colonization of both Hsr- mutant groups was not statistically different at 6 weeks post-infection ($p=0.1073$ for mutant 1, $p=0.2573$ for mutant 2) or at 9 weeks post-infection ($p=0.4686$ for mutant 1, $p=0.3565$ for mutant 2) from the ferret inoculated with the wild-type *H. mustelae* strain.

However, there was significantly less antral colonization in the mutant groups compared to the wild-type control ferret at 3 weeks ($p=0.0282$ for mutant 1, $p=0.0215$ for mutant 2) and at 12 weeks post-infection ($p=0.0034$ for mutant 1, $p=0.0040$ for mutant 2). In addition, the ferret that received the wild-type strain had robust colonization in its body at 12 weeks post-infection, but in the mutant groups, only one ferret had a few bacteria recovered in one of its body samples at the last timepoint. These results strongly support the hypothesis that the surface ring arrangement of *H. mustelae* impacts longterm, in vivo survival of the bacteria.

8/20 The Role of Th1 Immune Response in *Helicobacter heilmannii*-Associated Infection.

D.M.M. Queiroz[1], S.S. Cinque[1], R. Correa-Oliveira[2], M.M.D. Cabral[1], A.M.F. Nogueira[1], A.M.F. Nogueira[1], G.A. Rocha[1]. *[1]Universidade Federal de Minas Gerais: Belo Horizonte, Brazil; [2]Centro de Pesquisa Renè Rachou/Fiocruz: Belo Horizonte, Brazil*

We evaluated the role of the Th1 immune response in the *Helicobacter heilmannii* (Hh) infection.

Groups of 6 wild and IFN-gama knockout C57BL/6 mice were inoculated with gastric mucous of a Hh-positive swine. Six non-infected animals were also evaluated. At 4 weeks postinfection, the animals were sacrificed and the gastric mucosa were evaluated for the presence of Hh (urea test and carbolfuchsin stained smears) and histological abnormalities. To investigate the cellular response, splenocytes were cultured in the presence of *H. pylori* (ATCC 49503) crude extract and labeled with anti-CD4+, CD8+ and CD45RO+ clone B220+ (a B cell marker). The blastic cells were analysed by flow cytometry and the results were expressed as median percentage of blastic cells.

All inoculated mice were positive and uninfected mice were negative for Hh by all tests. A significant decrease of CD4+, CD8+ T cells and a significant increase of CD45RO+ B cells were observed in the Hh-positive knockout (12.7, 4.4 and 85.1 respectively) when compared with Hh-positive wild type mice (20.6 , 11.2 and 48.1; $p=0.02$, 0.002 and 0.002, respectively). No difference ($p>0.05$) was observed between Hh-infected knockout and non-infected wild mice (9.1, 3.7 and 87.6, respectively). The intensity of the gastric inflammation was also similar in Hh-infected knockout and Hh-negative wild mice. Conversely, the gastric inflammation was significantly less intense in the Hh-positive knockout animals than in the Hh-positive wild mice ($p=0.02$).

In conclusion, similarly to *H. pylori* infection, the Th1 response seems to be linked to gastric lesions associated to Hh infection.

8/21 Increased Gastric Emptying Induced by Vagotomy Associated with Pyloroplasty in Rats Chronically Infected with heilmannii type 1.

R.C. Barbuto, I. Duval-Araujo, D.M.M. Queiroz, C.D. Lima, A.E. Okawa. *Federal University of Minas Gerais: Belo Horizonte, Brazil*

The effect of surgeries for handling of chloridropeptic disease in *Helicobacter pylori*-infected organism in relation to gastric motility, hormonal secretion and possible adverse effects such as gastric cancer remains in the field of controversy for absence of an adequate experimental model. In the peptic ulcerous disease, as well as after troncular or gastric proximal vagotomy, some patients present basal and postprandial hypergastrinemy. The concurrence of the two conditions could be associate with increase of diarrhoea and increased gastric emptying observed in vagotomyzed patients, besides supplying an important mutagenic stimulation due to presence of enteric flora in stomach and duodenogastric reflux to gastric antrum. It was, then, objective of this study to evaluate the gastric emptying and gastrinemy in rats infected (Hh1+) or non-infected (Hh1-) with *Helicobacter heilmannii* type 1 (Hh1) and submitted (VP+) or not (VP-) to vagotomy with pyloroplasty. We studied 37 female Wistar rats divided into

following groups: VP-/Hh1- (n=7); VP+/Hh1- (n=8), VP-/Hh1+ (n=12) and VP+/Hh1+ (n=10). Thirty days after the inoculation of the bacterium, obtained by means gastric inoculation of 0.2 ml of mucus of stomach of a positive Hh1 swine, the animals had been submitted to a troncular vagotomy and pyloroplasty (VP+) or sham operation (VP-). Three months after surgical procedure, we made the study of gastric emptying by means scintigraphyc images using 99mTc-fitate diluted in peptone solution. After, the animals were killed and attainment of samples of blood for dosage of gastrin and samples of gastric mucous (antrum and body) to study gastric infection by means smears stained with carbolfuchsin, culture in BHM medium and blood agar. The statistical analysis had been made through Kruskall-Wallis test and Chi Square test, and differences considered for $p < 0.05$. The results showed an increase in gastric emptying index in VP+/Hh1+ animals when compared with other groups ($p < 0.01$). We observed increase in serum

gastrin level in VP+/Hh1- animals when compared with VP-/Hh1- (p=0.004) or VP-/Hh1+ (p=0.008), as well between VP+/Hh1+ and VP-/Hh1- (p=0.002) or VP-/Hh1+ (p=0.04). We observed also reduction in presence of Hh1 in gastric antrum of VP+/Hh1+ rats, and increase in gastric body localisation of bacteria in these animals. We concluding that vagotomy associated with pyloroplasty in Hh1-infected Wistar rats was responsible for an increase of gastric motility and hypergastrinemia, and may be associated also with migration of bacteria to the gastric body.

8/22 Characterization and Evaluation of the Native and Recombinantly-Expressed 19.3 kDa *Helicobacter pylori* Ferritin.

J. Fulginiti, M. Fiske, J. Caplan, M. Wetherell, S. Schmidt, D. Zhu, D. Dilts, P. Weidenborner, K. Belanger. *Wyeth Lederle Vaccines: West Henrietta, NY United States*

Objective: To characterize the biochemical and antigenic properties of the native and recombinant 19.3 kDa ferritin of *H. pylori* and evaluate the vaccine potential of these proteins in a mouse challenge model.

Methods: Purified native and recombinant proteins were characterized using SDS-PAGE, size exclusion chromatography, mass spectroscopy, and fluorescence spectroscopy. Antigenic similarities of native and recombinant proteins were evaluated in a Western blot assay. The *H. pylori* prophylactic mouse model was used to assess immunogenicity and ability of the native and recombinant proteins to reduce colonization of *H. pylori* strain SS1 in the gastric mucosa.

Results: Native and recombinant proteins are biochemically similar. Antibodies to the individual proteins are cross-reactive by Western blot analyses. When administered to C57BL/6 mice subcutaneously or orally, the native protein is able to significantly reduce colonization of SS1, whereas the recombinant protein does not. Protection from infection does not correlate to the level of circulating antibody as measured by ELISA.

Discussion: Despite the biochemical and antigenic similarities of the native and recombinant forms of the 19.3 kDa ferritin, the results of the mouse challenge studies suggest that the recombinant protein does not elicit important protective responses.

8/23 Antioxidant Food Supplementation Protects Against *H. pylori* Infection in Guinea Pigs.

H. Sjunnesson[1], E. Sturegård[1], R. Willén[2], T. Wadström[1]. [1]*Dept. Inf. Dis. and Med. Microbiol., Lund University: Lund, Sweden;* [2]*Dept. Pathol., Sahlgrens Hospital, University of Gothenburg: Gothenburg, Sweden*

Objective: Does antioxidants in diet decrease *Helicobacter pylori* growth and gastritis in guinea pigs?

Methods: *Experiment 1*: 38 *H. pylori* infected guinea pigs were given 3 different diets. (1) Control. (2) Vitamin A, C, E and selenium supplemented. (3) Vitamin C supplemented. Four uninfected animals were fed diet 1. *Experiment 2*: Group A, 7 animals given control diet, and group B, 8 animals given a diet supplemented with vitamins A, C, E, selenium and beta-carotene, were *H. pylori* infected. Four uninfected animals were fed control diet. Stomachs were cultured semi-quantitatively. *H. pylori* strains used were CagA positive. Animals were sacrificed 6 weeks post-inoculation. Gastritis was, by microscopy, graded 0(none)-3(severe) in H&E staining.

Results: *Experiment 1*: *H. pylori* was recovered from 43% of animals in the diet 1 group and from 16% and 42% of animals given diets 2 and 3. Mean antral gastritis in animals fed diets 1–3 was 0.93; 0.33 and 0.66. Uninfected control 0.25. Difference between diets 1 and 2 was not significant (P=0.065). *Experiment 2*: *H. pylori* was recovered from 100% in group A and 75% in B, with fewer colonies in B ($p < 0.05$). Mean gastritis in B was 2.25, compared to 2.57 in A (p=0.19) and 0.75 in controls ($p < 0.05$).

Discussion: Antioxidant supplementation reduces *H. pylori* growth in guinea pigs and together the two experiments point towards a decrease in the level of gastritis. These tentative results from the guinea pig model opens up possibilities for studying the role of diet in *H. pylori* related disease.

8/24 Influence of Murine *Helicobacter heilmannii* Type 1 Gastric Infection on the Production of Gastrin by G Cells in Vitro.

I. Duval-Araujo[1], D.M.M. Queiroz[2], N.T. Espírito-Santo[2], A.E. Okawa[2], A.H. Abjaud[2], P.H.D. Silva[2]. [1]*Federal University of Minas Gerais: Belo Horizonte, Brazil;* [2]*Federal University of Minas Gerais: Belo Horizonte, Brazil*

The chronic infection by gastric spiral bacteria has been associated, in human beings and animals, to an increase of serum gastrin levels in postprandial period. This event may be exacerbated by cholinergic extrinsic or intrinsic stimulation. In order to evaluate the secretory behaviour of G cell front the extrinsic (acetylcholine – Ach) or intrinsic (gastrin-release peptide – GRP) stimulation, we studied by means tissue culture the gastric antrum of 27 female wistar rats non-infected (control group – C) or infected (study group – E) during 90 days with *Helicobacter heilmannii* type 1 (Hh1). The gastric antrum of these animals were incubated during 30 minutes in McCoy nutritive solution (Sigma, USA) after addition of 0.5 ml of Ach in concentration of 1x10–3M (C1 and E1 groups) or GRP in concentration of 1x10–3M (C2 and E2 groups). After the first time of incubation, two samples of culture medium was collected and made new addition of 0.5 ml of Ach in concentration of 1x10–3M (C1 and E1 groups) or GRP in concentration of 1x10–3M (C2 and E2 groups). In the samples obtained in the first (gastA) or second (gastB) stimulation, we made the dosage of gastrin (pg/ml/100mg of tissue) and results compared through Student t test. In the first stimulation (gastA), it was observed, in study group, minor secretion of gastrin under stimulation of GRP when compared with Ach stimuli (p=0.02). After stimulation with Ach, gastric antrum of infected rats presented greater response to this agonist of that observed in gastric antrum of non-infected animals. After second stimuli, we observed that the secretory response of gastric antrum to Ach or GRP in infected animals was also exuberant. The data suggest that the interaction of vagal stimuli and acetylcholine secretion is, possible, an important factor in antral gastrin secretion in experimental model of spiral gastric bacteria infection.

8/25 Protection by therapeutic oral vaccination with inactivated bacteria in a mouse model *Helicobacter pylori* infection.

S. Raghavan, M. Hjulström, J. Holmgren, A.M. Svennerholm. *Gothenburg University: Gothenburg, Sweden*

Objective: Evaluate the protective effect of inactivated whole bacteria for therapeutic immunisation against experimental *H. pylori* infection.

Methods: Mouse adapted *H. pylori* strain SS1 bacteria was inactivated by mild formalin treatment. The inactivated bacteria retained different surface antigens, e.g. HpaA and urease. 6–8 week old C57BL/6 mice were infected and/ or immunized perorally 4 times at weekly intervals with $5X10^7$ inactivated bacteria, together with 10mug of cholera toxin as adjuvant. The mice were then divided into two groups: one was sacrificed one week after the last immunization and the other was treated for 5 days with a combination of antibiotics and omeprozol and subsequently reinfected one week later. The effect of therapeutic immunization with inactivated *H. pylori*, as well as the combined infection and immunisation against reinfection, was evaluated by determination of viable counts of *H. pylori* in the stomach of the immunized and reinfected mice respectively. The effect of initial infection alone in protection was also compared to reinfection.

Results: Therapeutic immunization with inactivated *H. pylori* SS1 gave significant protection when compared to unimmunized infected controls ($P < 0.001$). This protection was extended to reinfection ($P < 0.01$). In contrast, animals that were infected but not immunized were not protected against reinfection.

Conclusions: Inactivated *H. pylori* are strongly protective in therapeutic immunisation against *H. pylori* infection and also affords significant protection to reinfection.

8/26 Prophylactic Treatment of *H. pylori* Infection with Anti-*H. pylori* IgG-Antibodies.

L. Xu[1], F.O. Olfat[2], T. Boren[3], L. Engstrand[2], L. Hammarström[1].
[1]Division of Clinical Immunology , Huddinge Hospital, Karolinska Institute: Stockholm, Sweden; [2]The Swedish Institute for Infectious Disease Control: Stockholm, Sweden; [3]Department of Oral Biology, Umeå University: Umeå, Sweden

Objective: To analyze the feasibility of prophylactic treatment of *H. pylori* infection using passive immunization in the transgenic Lewis^b mouse model.

Methods: 10^9 cfu of *H. pylori* strain HP1 were incubated with/without bovine anti-*H. pylori* IgG immunoglobulins in 50mg/ml for 30 min at room temperature. Thirty 6-wk old mice were divided into 2 groups; an antibody treated group and a control group. 100µl of the bacterial suspension was given orally to the mice by gavage, once a week for two weeks. 10 days after the last treatment, animals were sacrificed and stomach tissue homogenates were cultured for *H. pylori*. The identity of *H. pylori* was confirmed by urease and immunoblot analyzes.

Results: The numbers of *H. pylori* in each group were calculated. A significant difference ($P < 0.05$, T-test.) was found between the antibody treated *H. pylori* animals versus the control group as shown in the table.

Groups	Infected (no)	*H. pylori* colonies/ mg of stomach tissue
Control group (n=15)	13	70.88
Antibody incubated group (n=15)	10	19.03*

* $P < 0.05$, T-test.

Conclusion: Our results suggest that prophylactic treatment with anti-*H. pylori* antibodies affect *H. pylori* infection. Further studies on how to increasing the effectiveness of the method are ongoing, i. e. using antibodies raised against selected/disease associated antigens such as the BabA adhesin.

0012992501 BIOSIS NO.: 200100164340
Identification of potential vaccine candidates from Helicobacter **pylori using proteomics technologies**
AUTHOR: Fiske M J (Reprint); Caplan J (Reprint); Wetherell M (Reprint); **Fulginiti J** (Reprint); Chakravarti D (Reprint
AUTHOR ADDRESS: Wyeth-Lederle Vaccines, West Henrietta, NY, USA**USA
JOURNAL: Gut 47 (Supplement 1): pA60 October, 2000 2000
MEDIUM: print
CONFERENCE/MEETING: XIIIth International Workshop on Gastroduodenal Pathology and Helicobacter pylori Rome, Italy October 11-14, 2000; 20001011
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RECORD TYPE: Citation
LANGUAGE: English

Identification of potential vaccine candidates from Helicobacter **pylori using proteomics technologies**
...AUTHOR: **Fulginiti J**
DESCRIPTORS:
ORGANISMS: **Helicobacter** pylori (Aerobic Helical or Vibrioid Gram-Negatives...

1/3,KWIC/3 (Item 3 from file: 5)
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0011907919 BIOSIS NO.: 199900167579
Intragastric immunization with recombinant H. pylori urease formulated with attenuated cholera toxin elicits systemic, mucosal and protective immune responses in C57BL/6 mice
AUTHOR: Zhu D; Schmidt S; **Fulginiti J** ; Fiske M; Phillips E; Peek J; Green B; Eldridge J H
AUTHOR ADDRESS: Wyeth-Lederle Vaccines, West Henrietta, NY 14586, USA**USA
JOURNAL: FASEB Journal 13 (4 PART 1): pA291 March 12, 1999 1999
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ISSN: 0892-6638
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RECORD TYPE: Citation
LANGUAGE: English

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Title: Identification of potential vaccine candidates from Helicobacter **pylori using proteomics technologies.**
Author(s): Fiske MJ; Caplan J; Wetherell M; **Fulginiti J** ; Chakravarti D
Corporate Source: WYETH LEDERLE VACCINES,W HENRIETTA//NY/
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Title: Identification of potential vaccine candidates from Helicobacter **pylori using proteomics technologies.**
Author(s): Fiske MJ; Caplan J; Wetherell M; **Fulginiti J** ; Chakravarti D

0250832 DBR Accession No.: 2000-05322 PATENT

Novel Helicobacter **pylori antigens useful for diagnostic and therapeutic purposes - recombinant surface antigenic protein production via vector plasmid-mediated gene transfer and expression in bacterium for bacterium infection recombinant vaccine**

AUTHOR: **Fulginiti J P** ; Fiske M J; Dilts D A

CORPORATE SOURCE: Madison, NJ, USA.

PATENT ASSIGNEE: American-Cyanamid 2000

PATENT NUMBER: WO 200000614 PATENT DATE: 20000106 WPI ACCESSION NO.: 2000-160674 (2014)

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LANGUAGE: English

Novel Helicobacter **pylori antigens useful for diagnostic and therapeutic purposes**

AUTHOR: **Fulginiti J P** ; Fiske M J; Dilts D A

ABSTRACT: **Helicobacter** pylori surface protein antigenic proteins (I), with mol.wt. of 75,000 (Ia) (708 amino...

DESCRIPTORS: **Helicobacter** pylori recombinant surface antigenic protein prep., vector plasmid-mediated gene transfer, expression in bacterium, antibody...

0733255

19 KILODALTON PROTEIN OF HELICOBACTER PYLORI

PROTEINE DE 19 KILODALTONS PRODUITE PAR LA BACTERIE HELICOBACTER PYLORI

Patent Applicant/Assignee:
AMERICAN CYANAMID COMPANY, Five Giralda Farms, Madison, NJ 07940, US, US (Residence), US (Nationality), (For all designated states except: US)
Patent Applicant/Inventor:
FISKE Michael, 167 Wood Run, Rochester, NY 14612, US, US (Residence), US (Nationality), (Designated only for: US)
ZHU Duzhang, 50 Brittany Circle, Rochester, NY 14618, US, US (Residence), US (Nationality), (Designated only for: US)
FULGINTI James P, 5180 Foster Road, Canadaigua, NY 14424, US, US (Residence), US (Nationality), (Designated only for: US)
SCHMIDT Susan G, 455 Eastbrooke Lane, Rochester, NY 14618, US, US (Residence), US (Nationality), (Designated only for: US)
Legal Representative:
WEBSTER Darryl L, American Home Products Corporation, Patent Law Dept. 2B2, One Campus Drive, Parsippany, NJ 07054, US

Patent and Priority Information (Country, Number, Date):
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Application: WO 2000US2938 20000203 (PCT/WO US0002938)
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Detailed Description

ORAL IMMUNIZATION OF MICE WITH LIVE ATTENUATED *SALMONELLA TYPHIMURIUM* EXPRESSING *HELICOBACTER PYLORI* UREASE.

J. Fulginiti, D. Zhu, S. Schmidt, P. Weidenborner, D. Lane, R. Deich and J.H. Eldridge. Lederle-Praxis Biologicals, West Henrietta, NY 14586

Attenuated *Salmonella* expressing foreign genes are attractive for use as oral vaccine carriers. We studied systemic and mucosal immune responses of BALB/c mice to *Helicobacter pylori* urease expressed in *Salmonella aroA* vaccine strain SL3261 following oral administration. Groups of eight-week old BALB/c mice were immunized intragastrically (IG) with 10^{10} *S. typhimurium aroA* transformed with plasmid pPX5024, which expresses *H. pylori* urease, on days 0, 2, 14 and 16. Control groups were immunized with 50 µg of purified native urease admixed with cholera toxin (CT), 10^6 SL3261/pPX5024 intraperitoneally (IP), or not vaccinated. Tissues were examined for the presence of the vaccine strain on days 1, 7 and 28. Two weeks after the last immunization, serum and bronchoalveolar washes (BAW) were collected to allow measurement of serum and mucosal IgA and IgG antibodies specific to urease in an ELISA. Results showed that plasmid-containing bacteria were recovered from the Peyer's patches, livers and spleens of IG immunized animals on all days examined. Both serum and BAW anti-urease antibodies were present after oral administration, but only serum antibodies were elicited by IP immunization. Mice which were orally immunized with 50 µg of purified native urease together with 10 µg of the adjuvant CT mounted both serum IgG and BAW IgA responses. However, oral immunization with *H. pylori* urease adjuvanted with CT induced a predominant IgG1 response, whereas *Salmonella* urease constructs preferentially induced an IgG2a response. Studies are underway to characterize the immune response which correlates with protection in the murine *Helicobacter felis* challenge model.

04446567 Supplier Number: 46526912 (THIS IS THE FULLTEXT)

Vaccine Development "Vaccines of Tomorrow: The Hopes of Science."

Infectious Disease Weekly, pN/A
July 8, 1996
ISSN: 1078-2850
Language: English Record Type: Fulltext
Document Type: Newsletter; Professional Trade
Word Count: 427

TEXT:

J.P. Krachenbuhl. Institute of Experimental Cancer Research and Institute of Biochemistry, University of Lausanne, France.

According to an abstract submitted by the authors to the 4th International Conference on the Prevention of Infection, held May 6-7, 1996, in Nice, France, "The ideal vaccine as defined by WHO should have the following properties: cost effectiveness ((approx)50 cents a dose) long lasting protection, mucosal administration as a unique dose given preferentially in the first year of age, and no requirement for a cold chain. This is a real challenge for the scientists. Recombinant DNA technology linked to a better understanding of host-microbial interactions and molecular and cellular mechanisms of the host response have revolutionized the field of vaccinology and should contribute to the design of ideal vaccines. In mucosal vaccinology, however, progress has been hampered by the difficulty to deliver vaccine carriers by the oral route and by difficulties in assessing immune responses in the mucosal environment. Many microorganisms invade and infect the host via mucosal surfaces by crossing the tight epithelial barrier of the gut, the airways or the urogenital tract. They usually exploit the antigen sampling cells of the mucosal immune system. Resident specialized epithelial cells, the so-called M cells present in the follicle-associated epithelium of mucosa-associated lymphoid tissue act as a portal of entry for pathogenic viruses, bacteria and parasites. In stratified and simple epithelia, uptake of microorganisms is mediated by non epithelial cells, the dendritic of Langerhans cells that are to migrate to local or distant lymphoid tissue, thus facilitating systemic spread of the infectious agents. Theses considerations are important for the design of mucosal vaccines. Vaccines that have to be administered by mucosal routes (oral, nasal, rectal or vaginal) have to reach the antigen sampling sites, cross the epithelial barrier, and enter lymphoid tissue where they can be seen by the immune system. We have designed a subunit vaccine against a pathogen. **Helicobacter pylori** responsible for chronic atrophic gastritis in humans, ulcer disease, and gastric carcinomas and lymphomas. **Helicobacter pylori** survives in the stomach due to its **urease** activity. Recombinant **urease** and cholera or labile toxin as mucosal adjuvant, administered orally elicit an immune response which is both prophylactic and therapeutic in mice. The vaccine is safe in humans and a phase 2 trial with infected volunteers is currently underway in Lausanne. A second generation of vaccine aimed at enhancing the duration of protection has been designed. We have selected live **recombinant Salmonella typhimurium** as a vaccine carrier **attenuated** in its survival in macrophages. The bacterial vaccine is presently tested in mice."

PUBLISHER NAME: Charles W. Henderson
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GEOGRAPHIC NAMES: *4EUFR (France)
PRODUCT NAMES: *2831210 (Vaccines for Human Use)
INDUSTRY NAMES: BUSN (Any type of business); HLTH (Healthcare - Medical and Health)
NAICS CODES: 325412 (Pharmaceutical Preparation Manufacturing)

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L2: Entry 1 of 1 File: PGPB Nov 22, 2001

DOCUMENT-IDENTIFIER: US 20010044416 A1
TITLE: Immunostimulatory nucleic acids for inducing a Th2 immune response

Detail Description Paragraph:
[0125] It was discovered according to the invention that Th2 immunostimulatory nucleic acids induced predominantly Th2-like responses (IgG1>>IgG2a), whereas CpG nucleic acids resulted in mixed Th1/Th2 or predominantly Th1-like responses. Th2 responses in some instances are also considered mixed immune response that are nonetheless biased towards a Th2 profile. Th2 responses are highly desirable for the prevention or treatment of a number of Th1-mediated diseases including: organ-specific autoimmune disorders, Crohn's disease, Helicobacter pylori-induced peptic ulcer, acute solid organ allograft rejection, and unexplained recurrent abortion. The only adjuvant currently licensed for use in humans in most countries of the world, including the USA, is aluminum hydroxide (alum) which, although having a Th2 immunostimulatory effect, is weak, is associated with undesirable local tissue reactions, and is generally considered unsuitable for mucosal delivery. CT, which also enhances Th2-like immune responses, can be given mucosally, however it is too toxic for use in humans. A mouse (.about.20 g body weight) can tolerate the toxic effects of up to 10 .mu.g of CT, however a dose as little as 1-5 .mu.g will cause severe diarrhea in a human (.about.70 kg body weight) (Jertborn et al., 1992). Animals receiving Th2 immunostimulatory nucleic acids showed no short-term signs of distress over those receiving antigen alone, and all recovered quickly with no apparent long-lasting effects even with doses of up to 500 .mu.g. This is the first report of mucosal application of Th2 immunostimulatory nucleic acids to augment immune responses and the Th2-bias of the responses induced by Th2 immunostimulatory nucleic acids is of great importance in the development of effective Th2 biased prophylactic or therapeutic strategies.

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          53718  SALMONEL?
      S1     23  FULGINITI AND SALMONEL?
?rd
```

>>>Duplicate detection is not supported for File 349.
>>>Duplicate detection is not supported for File 348.
>>>Duplicate detection is not supported for File 229.
>>>Duplicate detection is not supported for File 654.

>>>Records from unsupported files will be retained in the RD set.
...completed examining records
      S2   19 RD (unique items)
?t s2/3,kwic/all

2/3,KWIC/1   (Item 1 from file: 349)
DIALOG(R)File 349:PCT FULLTEXT

00733255
19 KILODALTON PROTEIN OF HELICOBACTER PYLORI
PROTEINE DE 19 KILODALTONS PRODUITE PAR LA BACTERIE HELICOBACTER PYLORI
Patent Applicant/Assignee:
  AMERICAN CYANAMID COMPANY, Five Giralda Farms, Madison, NJ 07940, US, US (Residence), US (Nationality), (For all designated states except: US)
Patent Applicant/Inventor:
  FISKE Michael, 167 Wood Run, Rochester, NY 14612, US, US (Residence), US (Nationality), (Designated only for: US)
  ZHU Duzhang, 50 Brittany Circle, Rochester, NY 14618, US, US (Residence), US (Nationality), (Designated only for: US)
  FULGINTI James P, 5180 Foster Road, Canadaigua, NY 14424, US, US (Residence), US (Nationality), (Designated only for: US)
  SCHMIDT Susan G, 455 Eastbrooke Lane, Rochester, NY 14618, US, US (Residence), US (Nationality), (Designated only for: US)
Legal Representative:
  WEBSTER Darryl L, American Home Products Corporation, Patent Law Dept. 2B2, One Campus Drive, Parsippany, NJ 07054, US
Patent and Priority Information (Country, Number, Date):
  Patent: WO 200046242 A2 20000810 (WO 0046242)
  Application: WO 2000US2938 20000203 (PCT/WO US0002938)
  Priority Application: US 99118631 19990204
Designated States: AE AL AM AT AU AZ BA BB BG BR BY CA CH CN CR CU CZ DE DK DM EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG US UZ VN YU ZA ZW
  (EP) AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE
  (OA) BF BJ CF CG CI CM GA GN GW ML MR NE SN TD TG
  (AP) GH GM KE LS MW SD SL SZ TZ UG ZW
  (EA) AM AZ BY KG KZ MD RU TJ TM
Publication Language: English
Filing Language: English
Fulltext Word Count: 9731

Fulltext Availability:
  Detailed Description
  Claims

Detailed Description
... immunogenic fragment as a foreign polypeptide. Particularly, bacteria that colonize the gastrointestinal tract, such as **Salmonella** , Shigella, Yersinia, Vibrio, Escherichia and BCG have been developed as vaccine vectors, and these and...

Claim
... FlGw3
  SUBSTITUTE SHEET (RULE 26)
  SEQUENCE LISTING
  <110> Fiske, Michael
  Zhu, Duzhang
  Schmidt, Susan G
   Fulginiti , James P
  <120> 19 Kilodalton Protein of Helicobacter Pylori
  <130> 33537PCT
  <140>

<141>
<150> 60...

2/3,KWIC/2 (Item 2 from file: 349)
DIALOG(R)File 349:PCT FULLTEXT

00537241
NOVEL ANTIGENS OF i(HELICOBACTER PYLORI)
NOUVEAUX ANTIGENES D'i(HELIOBACTER PYLORI)
Patent Applicant/Assignee:
AMERICAN CYANAMID COMPANY,
FULGINITI James Peter,
FISKE Michael James,
DILTS Deborah Ann
Inventor(s):
FULGINITI James Peter,
FISKE Michael James,
DILTS Deborah Ann,
Patent and Priority Information (Country, Number, Date):
Patent: WO 200000614 A2 20000106 (WO 0000614)
Application: WO 99US14375 19990625 (PCT/WO US9914375)
Priority Application: US 9890851 19980626
Designated States: AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES FI GB GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG US UZ VN YU ZA ZW GH GM KE LS MW SD SL SZ UG ZW AM AZ BY KG KZ MD RU TJ TM AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE BF BJ CF CG CI CM GA GN GW ML MR NE SN TD TG
Publication Language: English
Fulltext Word Count: 22776

Patent Applicant/Assignee:
... **FULGINITI** James Peter
Fulltext Availability:
Detailed Description

Detailed Description
... immunogenic fragment as a foreign polypeptide. Particularly, bacteria that colonize the gastrointestinal tract, such as **Salmonella** , Shigella, Yersinia, Vibrio, Escherichia and BCG have been developed as vaccine vectors, and these and...

2/3,KWIC/3 (Item 3 from file: 349)
DIALOG(R)File 349:PCT FULLTEXT

00411110
MUTATED ANTIBODY-DEPENDENT INFECTION ENHANCING DOMAINS OF HIV
MUTATIONS DANS LES DOMAINES FACILITANT ANTICORPS-DEPENDANT D'INFECTION DU VIH
Patent Applicant/Assignee:
VANDERBILT UNIVERSITY,
MITCHELL William M,
Inventor(s):
MITCHELL William M,
Patent and Priority Information (Country, Number, Date):
Patent: WO 9801570 A2 19980115
Application: WO 97US11667 19970702 (PCT/WO US9711667)
Priority Application: US 9621668 19960705
Designated States: AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES FI GB GE GH HU IL IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG US UZ VN YU ZW GH KE LS MW SD SZ UG ZW AM AZ BY KG KZ MD RU TJ TM AT BE CH DE DK ES FI FR GB GR IE IT LU MC NL PT SE BF BJ CF CG CI CM GA GN ML MR NE SN TD TG
Publication Language: English

Fulltext Word Count: 22801
Fulltext Availability:
Detailed Description

Detailed Description
... such as E. coli (e.g., E. coli
K12 strains, SLreptomyces, Pseudomonas, Serratia marcescens
and **Salmonella** typhimurium, insect cells (baculovirus),
including Drosophila, fungal cells, such as yeast cells,
plant cells and...peritonitis virus
challenge due to recombinant vaccinia virus
immunization. J Virol 1990, 64:1407
27. **Fulginiti** VA, Eller JJ, Downie AW, Kempe CH.

Altered reactivity to measles virus: atypical
measles in...

...trivalent
parainfluenza virus vaccine in a pediatric
population. Am J Epidemol 1969, 89:449
28. **Fulginiti** VA, Eller JJ, Sieber OF, joyner JW,
Minamitani M, Meiklejohn G. Respiratory virus
immunization 1...

2/3,KWIC/4 (Item 4 from file: 349)
DIALOG(R)File 349:PCT FULLTEXT

00373699
HORMONE IMMUNOMODULATED INDUCTION OF MUCOSAL IMMUNE RESPONSES
INDUCTION HORMONALE IMMUNOMODULEE DE REPONSES IMMUNITAIRES DES MUQUEUSES
Patent Applicant/Assignee:
MERLIN TECHNOLOGIES INC,
Inventor(s):
MITCHELL William M,
Patent and Priority Information (Country, Number, Date):
Patent: WO 9714442 A1 19970424
Application: WO 96US16845 19961017 (PCT/WO US9616845)
Priority Application: US 95544575 19951018
Designated States: AU CA JP AT BE CH DE DK ES FI FR GB GR IE IT LU MC NL PT
SE
Publication Language: English
Fulltext Word Count: 24434

Fulltext Availability:
Detailed Description

Detailed Description
... Examples of bacterial pathogens include, but are not
limited to, species of the following genera: **Salmonella** ,
Shigella, Chlamydia, Helicobacter, Yersinia, Bordatella,
Pseudomonas, Neisseria, Vibrio and Haemophilus, among
others.

Antigen-Encoding DNA...trivalent parainfluenza
virus vaccine in a pediatric population. Am J Epidemiol
1969, 89:449
128. **Fulginiti** VA, Eller JJ, Sieber OF, Joyner JW,
Minamitani M, and Meiklejohn G. Respiratory virus
immunization...rats by prior intramuscular inoculation of formalin
inactivated virus. J Virol 1986, 57:721
132. **Fulginiti** VA, Eller JJ, Downie AW, and Kempe
CH, Altered reactrivity to measles virus: atypical
measles...

2/3,KWIC/5 (Item 5 from file: 349)
DIALOG(R)File 349:PCT FULLTEXT

00338844
METHODS AND COMPOSITIONS FOR INDUCING MUCOSAL IMMUNE RESPONSES
PROCEDES ET COMPOSITIONS DESTINES A INDUIRE DES REPONSES IMMUNITAIRES DES MUQUEUSES
Patent Applicant/Assignee:
VANDERBILT UNIVERSITY,
Inventor(s):
MITCHELL William M,
Patent and Priority Information (Country, Number, Date):
Patent: WO 9621356 A1 19960718
Application: WO 95US8374 19950703 (PCT/WO US9508374)
Priority Application: US 95372429 19950113
Designated States: AU CA JP
Publication Language: English
Fulltext Word Count: 21095

Fulltext Availability:
Detailed Description

Detailed Description
... Examples of bacterial pathogens include, but are not limited to, species of the following genera: **Salmonella** , Shigella, Chlamydia, Helicobacter,, Yersinia,, Bordatella, Pseudomonas, Neisseria, Vibrio and Haemophilus, among others.

Antigen-Encoding DNA...trivalent parainfluenza virus vaccine in a pediatric population. Am J Epidemiol 1969, 89:449463.

128. **Fulginiti** VA, Eller JJ, Sieber OF, Joyner JW, Minamitani M, and Meiklejohn G. Respiratory virus immunization...

...rats by prior intramuscular inoculation of formalin inactivated virus. J Virol 1986, 57:721
132. **Fulginiti** VA, Eller JJ, Downie AW, and Kempe CH.

Altered reactivity to measles virus: atypical measles...

2/3,KWIC/6 (Item 6 from file: 349)
DIALOG(R)File 349:PCT FULLTEXT

00327890
GENE THERAPY VECTORS AND VACCINES BASED ON NON-SEGMENTED NEGATIVES STRANDED RNA VIRUSES
VECTEURS DE THERAPIE GENIQUE ET VACCINS BASES SUR DES VIRUS A ARN A BRINS NEGATIFS NON SEGMENTES
Patent Applicant/Assignee:
THE UAB RESEARCH FOUNDATION,
Inventor(s):
WERTZ Gail W,
YU Qingzhong,
BALL Laurence A,
BARR John N,
WHELAN Sean P J,
Patent and Priority Information (Country, Number, Date):
Patent: WO 9610400 A1 19960411
Application: WO 95US12507 19950929 (PCT/WO US9512507)
Priority Application: US 94316438 19940930; US 95245587 19950607
Designated States: CA JP AT BE CH DE DK ES FR GB GR IE IT LU MC NL PT SE
Publication Language: English
Fulltext Word Count: 22317
Fulltext Availability:
Detailed Description
Claims

Detailed Description
... enhanced frequency and severity of lower respiratory tract disease in children exposed to subsequent reinfection (**Fulginiti** , V.A. et. al., (I 969) American Journal ofEpidemiology 89,435-448 and Kim, H...0009000 Great
syncytial virus infections, pneumonia (1601000+)
Rotavirus Diarrhea, dehydration 1401000@000 Great
(873,000+)
Salmonella typhi Typhoid fever (with platelet 30,000,000 Small bacterium and intestinal damage (581,000...

Claim
... group consistiniz of intestinal toxin woducine E coli. Hemophilus influenza tvr)e b. Neisseria meningitidis, **Salmonella** typhi, Shigella, Streptococcus Group A, Streptococcus pneumoniae, and Vibrio cholerae.

12 A vaccine of claim...the group consisting of intestinal toxin producing E coli, Hemophilus influenza type b, Neisseria meningitidis, **Salmonella** typhi, Shigella, Streptococcus Group A, Streptococcus pneumoniae, and Vibrio cholerae.

29 A vaccine of claim...

2/3,KWIC/7 (Item 1 from file: 148)
DIALOG(R)File 148:Gale Group Trade & Industry DB
(c)2004 The Gale Group. All rts. reserv.

09941102 SUPPLIER NUMBER: 20069193 (USE FORMAT 7 OR 9 FOR FULL TEXT)
Influence of disease burden, public perception, and other factors on new vaccine development, implementation, and continued use.
Levine, Myron M.; Levine, Orin S.
Lancet, v350, n9088, p1386(7)
Nov 8, 1997
ISSN: 0099-5355 LANGUAGE: English RECORD TYPE: Fulltext; Abstract
WORD COUNT: 6551 LINE COUNT: 00543

... leading to dehydration particularly in cool climates.
For some pathogens, such as measles virus or **Salmonella** typhi, only a single relevant strain or serotype exists and years of vaccine use has... immunized with an Hib con- jugate vaccine. J Infect Dis 1991; 164: 982-86.
41 **Fulginiti** VA, Eller JJ, Downie AW, Kempe CH. Altered reactivity to measles virus. Atypical measles in...

2/3,KWIC/8 (Item 2 from file: 148)
DIALOG(R)File 148:Gale Group Trade & Industry DB
(c)2004 The Gale Group. All rts. reserv.

08271719 SUPPLIER NUMBER: 17460638 (USE FORMAT 7 OR 9 FOR FULL TEXT)
Trends of diarrheal disease - associated mortality in US children, 1968 through 1991.
Kilgore, Paul E.; Holman, Robert C.; Clarke, Matthew J.; Glass, Roger I.
JAMA, The Journal of the American Medical Association, v274, n14, p1143(6)
Oct 11, 1995
ISSN: 0098-7484 LANGUAGE: English RECORD TYPE: Fulltext; Abstract
WORD COUNT: 4113 LINE COUNT: 00338

... by bacteria (5.1%) and parasites (0.3%). Among the deaths associated with bacterial agents, **Salmonella** organisms were more commonly identified in infants, whereas Shigella organisms were more often identified in...Methods. Boston, Mass: PWS-Kent Publishing Company; 1988.
(18.) Klein JO, Brunell PA, Cherry JD, **Fulginiti** VA. Report of the Committee an Infectious Diseases, Redbook. 19th ed. Evanston, Ill: American Academy...

2/3,KWIC/9 (Item 3 from file: 148)

DIALOG(R)File 148:Gale Group Trade & Industry DB
(c)2004 The Gale Group. All rts. reserv.

06759376 SUPPLIER NUMBER: 14480184 (USE FORMAT 7 OR 9 FOR FULL TEXT)
Major changes seen for US childhood vaccination. (Medical News & Perspectives)
Marwick, Charles
JAMA, The Journal of the American Medical Association, v270, n15, p1782(2)
Oct 20, 1993
ISSN: 0098-7484 LANGUAGE: ENGLISH RECORD TYPE: FULLTEXT; ABSTRACT
WORD COUNT: 1377 LINE COUNT: 00114

... s important to think ahead, "he says.
The chair of the advisory committee, Vincent A. **Fulginiti** , MD (who was until last month editor of the American Medical Association's American Journal...

...Human Services] on future directions in terms of what vaccines might be given higher priority."
Fulginiti also suggests that financial incentives may encourage the development of needed vaccines that might not...

...Pediatrics and the CDC's Immunization Practices Committee, but to face the future.
For example, **Fulginiti** says that "the coming combination vaccines are going to pose some real issues for this...Development of vaccines - primarily for use in developing countries - against such diseases as shigella, cholera, **salmonella** , malaria, and dengue.
* Improvements in existing vaccines; for example, the development of a heat-stable...

...or that are otherwise unprofitable.
Authority Already Here?
Making what he called "a bold move," **Fulginiti** suggested that the National Vaccine Program Office was in an ideal position to become the...

...therefore, "we have already, in fact, a national vaccine authority." If his suggestion is viable, **Fulginiti** says, legislation can be devised to grant it broad-based authority.
In general, the committee...

2/3,KWIC/10 (Item 1 from file: 348)
DIALOG(R)File 348:EUROPEAN PATENTS
(c) 2004 European Patent Office. All rts. reserv.

00856305
INDUCTION OF MUCOSAL IMMUNE RESPONSES BY VITAMIN D3 AND ANTIGEN-ENCODING DNA COMPLEXE TO CATIONIC LIPID
INDUKTION VON IMMUNANTWORTEN DER SCHLEIMHAUTE DURCH VERABREICHUNG VON VITAMINE D3 UND DNS, DIE FUR EIN ANTIGEN KODIERT UND DIE MIT MIT EINEM KATIONISCHEN LIPID EIN COMPLEX BILDET.
INDUCTION DE L'IMMUNITE MUCOSALE PAR L'ADMINISTRATION DE LA VITAMINE D3 ET D'ADN CODANT UN ANTIGENE COMPLEXE A UN LIPIDE CATIONIQUE
PATENT ASSIGNEE:
VANDERBILT UNIVERSITY, (554287), 405 Kirkland Hall, Nashville, TN 37240, (US), (Proprietor designated states: all)
INVENTOR:
MITCHELL, William, M., 251 Vaughns Gap Road, Nashville, TN 37205, (US)
LEGAL REPRESENTATIVE:
Price, Vincent Andrew et al (79513), Fry Heath & Spence LLP The Gables Massetts Road, Horley Surrey RH6 7DQ, (GB)
PATENT (CC, No, Kind, Date): EP 855919 A1 980805 (Basic)
EP 855919 B1 030723
WO 97014442 970424
APPLICATION (CC, No, Date): EP 96936786 961017; WO 96US16845 961017
PRIORITY (CC, No, Date): US 544575 951018
DESIGNATED STATES: AT; BE; CH; DE; DK; ES; FI; FR; GB; GR; IE; IT; LI; LU; MC; NL; PT; SE

INTERNATIONAL PATENT CLASS: A61K-048/00; A61K-031/59; A61K-047/18; A61K-047/28
NOTE:
No A-document published by EPO
LANGUAGE (Publication,Procedural,Application): English; English; English
FULLTEXT AVAILABILITY:

Available Text	Language	Update	Word Count
CLAIMS B	(English)	200330	164
CLAIMS B	(German)	200330	180
CLAIMS B	(French)	200330	179
SPEC B	(English)	200330	20415
Total word count - document A			0
Total word count - document B			20938
Total word count - documents A + B			20938

...SPECIFICATION Examples of bacterial pathogens include, but are not limited to, species of the following genera: **Salmonella** , Shigella, Chlamydia, Helicobacter, Yersinia, Bordatella, Pseudomonas, Neisseria, Vibrio and Haemophilus, among others.
Antigen-Encoding DNA...parainfluenza virus vaccine in a pediatric population. Am J Epidemiol 1969, 89:449-463.
128. **Fulginiti** VA, Eller JJ, Sieber OF, Joyner JW, Minamitani M, and Meiklejohn G. Respiratory virus immunization...

...by prior intramuscular inoculation of formalin-inactivated virus. J Virol 1986, 57:721-728.
132. **Fulginiti** VA, Eller JJ, Downie AW, and Kempe CH. Altered reactrivity to measles virus: atypical measles...

2/3,KWIC/11 (Item 2 from file: 348)
DIALOG(R)File 348:EUROPEAN PATENTS
(c) 2004 European Patent Office. All rts. reserv.

00566187
Respiratory syncytial virus (RSV) mutant and pharmaceutical composition containing such a mutant
Mutante des Respiratory-syncytial-Virus (RS-Virus) und eine eine solche Mutante enthaltende pharmazeutische Zusammensetzung
Mutant du virus syncytial respiratoire (RSV) et composition pharmaceutique contenant un tel mutant
PATENT ASSIGNEE:
AMERICAN CYANAMID COMPANY, (212591), 1937 West Main Street P.O. Box 60, Stamford Connecticut 06904-0060, (US), (applicant designated states: AT;BE;CH;DE;DK;ES;FR;GB;GR;IE;IT;LI;LU;NL;PT;SE)
INVENTOR:
Randolph, Valerie Bruce, 535 Pine Brook Road, Lincoln Park, New Jersey 07035, (US)
Crowley, Joan Coflan, 261 Glenwood Road, Englewood, New Jersey 07631, (US)
LEGAL REPRESENTATIVE:
Wachtershauser, Gunter, Prof. Dr. (12711), Patentanwalt, Tal 29, 80331 Munchen, (DE)
PATENT (CC, No, Kind, Date): EP 567100 A1 931027 (Basic)
EP 567100 B1 990317
APPLICATION (CC, No, Date): EP 93106496 930421;
PRIORITY (CC, No, Date): US 871420 920421
DESIGNATED STATES: AT; BE; CH; DE; DK; ES; FR; GB; GR; IE; IT; LI; LU; NL; PT; SE
INTERNATIONAL PATENT CLASS: C12N-015/45; C12N-007/08; C12N-015/87; A61K-048/00; C12N-015/86; A61K-039/155; C07K-014/135;
ABSTRACT WORD COUNT: 77

LANGUAGE (Publication,Procedural,Application): English; English; English
FULLTEXT AVAILABILITY:

Available Text	Language	Update	Word Count
CLAIMS B	(English)	9911	275
CLAIMS B	(German)	9911	215
CLAIMS B	(French)	9911	308

```
      SPEC B    (English)  9911      10058
Total word count - document A           0
Total word count - document B       10856
Total word count - documents A + B  10856
```

...SPECIFICATION illness following subsequent natural infection with RSV (Kapikian et al, 1969; Kim et al, 1969; **Fulginiti** et al, 1969; Chin et al; 1969). The reasons why this vaccine enhanced RSV disease... polypeptides by a live virus or bacterial vector (e.g. baculovirus, vaccinia virus, adenovirus, attenuated **Salmonella**), (5) as viral vectors for expression of immunogenic proteins from other viruses, e.g., RSV...grown RS virus in adult volunteers. Journal of the American Medical Association 204:690-694.

Fulginiti VA, Eller JJ, Sieber OF, Joyner JW, Minamitani M, Meiklejohn G (1969): I. A field...

2/3,KWIC/12 (Item 3 from file: 348)

DIALOG(R)File 348:EUROPEAN PATENTS
(c) 2004 European Patent Office. All rts. reserv.

00517216

Stable pura vectors and uses thereof
Stabile pura-Vektoren und ihre Verwendung
Vecteurs pura stables et leur utilisation

PATENT ASSIGNEE:
AMERICAN CYANAMID COMPANY, (212594), One Cyanamid Plaza, Wayne, NJ 07470-8426, (US), (Proprietor designated states: all)

INVENTOR:
Brey III, Robert Newton, 74 Sagamore Drive, Rochester, New York 14617, (US)
Fulginiti , James Peter, 5180 Foster Road, Canandaigua, New York 14424, (US)
Anilionis, Algis, 25 Whistlerhill Lane, Huntington, New York 11743, (US

LEGAL REPRESENTATIVE:
Wachtershauser, Gunter, Prof. Dr. (12711), Patentanwalt, Tal 29, 80331 Munchen, (DE)

PATENT (CC, No, Kind, Date): EP 512260 A2 921111 (Basic)
EP 512260 A3 930728
EP 512260 B1 010704
APPLICATION (CC, No, Date): EP 92105887 920406;
PRIORITY (CC, No, Date): US 695706 910503
DESIGNATED STATES: AT; BE; CH; DE; DK; ES; FR; GB; GR; IT; LI; LU; NL; PT; SE
INTERNATIONAL PATENT CLASS: C12N-015/74; A61K-039/112; C12N-015/74; C12R-1:42
ABSTRACT WORD COUNT: 92
NOTE:
Figure number on first page: NONE

LANGUAGE (Publication,Procedural,Application): English; English; English
FULLTEXT AVAILABILITY:

Available Text	Language	Update	Word Count
CLAIMS A	(English)	EPABF1	514
CLAIMS B	(English)	200127	1730
CLAIMS B	(German)	200127	1667
CLAIMS B	(French)	200127	2184
SPEC A	(English)	EPABF1	8964
SPEC B	(English)	200127	8872

Total word count - document A 9479
Total word count - document B 14453
Total word count - documents A + B 23932

INVENTOR:
... US)
Fulginiti , James Peter...

...ABSTRACT is useful in production organisms during fermentation and in live vaccine bacteria, such as attenuated **Salmonella** typhi. This system

allows for selection of chromosomal integrants and for selection and stable plasmid...

...SPECIFICATION by the pSC101 par locus. Other partition regions, such as a partition region from a **Salmonella** typhimurium virulence plasmid, have also been used successfully to stabilize cloning vehicles.

Although cloning vehicles...

...lead to plasmid stabilization. Complementation of a chromosomal mutation for aspartic semialdehyde dehydrogenase (asd) in **Salmonella** typhimurium or D-alanine racemase mutation (dal) in Bacillus subtilis, which each lead to faulty...CS fusion protein (pPX3007).

Figure 7 shows the results of in vivo stabilization studies of **Salmonella** typhimurium strains by purA complementation. S. typhimurium recovered from A: spleens, B: livers and C...

...purA gene product). The purA locus is especially important in the design of live attenuated **Salmonella** and related enteric vaccine vectors since the presence of purA mutations on the chromosome leads...

...or vitamin precursors (such as folic acid) which are dependent upon aromatic compound biosynthesis. Thus, **Salmonella** typhimurium harboring deletion mutations in both aroA and in purA are ineffective in inducing a ...

...defects in aromatic biosynthetic genes (such as aroA) are effective in inducing immune responses. Although **Salmonella** harboring aroA mutations are able to replicate to a limited extent intracellularly, those containing additional...be used in the vaccine formulations of the invention include but are not limited to **Salmonella** spp., invasive E. coli (EIEC), and Shigella spp. In a preferred embodiment, invasive bacteria which reside in lymphoid tissues such as the spleen (e.g., **Salmonella** spp.) are used. Such bacteria can invade gut epithelial tissue and/or Peyer's patches...

...Cold Spring Harbor, New York), laboratory selection of natural mutations, etc. Methods for obtaining attenuated **Salmonella** strains which are non-reverting non-virulent auxotrophic mutants suitable for use as live vaccines...

...are incorporated by reference herein in their entirety. A reliable method to achieve attenuation of **Salmonella** has also been described (S.K. Hoiseth, and B.A.D. Stocker, Nature, 291:238...

...53:47 (1982)) and can be used in a particular embodiment of the invention.

Attenuated **Salmonella** which can be used in the live vaccine formulations of the invention include but are...

...those species listed in Table 1 below. (see image in original document)

In specific embodiments, **Salmonella** bacteria that have been attenutated by chromosomal deletion of gene(s) for aromatic compound biosynthesis...

...equivalent to typhoid fever. galE mutants which can used include but are not limited to **Salmonella** typhi strains Ty21a (Germanier, Bacteria Vaccines, Academic Press, NY pp. 137-165) **Salmonella** typhimurium G30D, etc.

Expression of Gene Products and Uses

The invention also pertains to methods...encoding antigens derived from pathogenic bacterial, viral or parasitic sources can be introduced into attenuated **Salmonella** typhi for use as live vaccines in humans, to protect against, for example, typhoid fever...

...diseases and sexually transmitted diseases including AIDS. Alternatively, such genes can be introduced into other **Salmonella** capable of infecting animal species, e.g., S. dublin for use as live attenuated cattle...

...added at 100 (mu)g/ml.

Genetic Transformation and Transduction

Plasmids were introduced directly into **Salmonella** by electroporation with a BTX Transfector 100 with the 0.5 mm electrode and a...York (1982)). Plasmid constructions were isolated and characterized first in E. coli, before transferring to **Salmonella** spp., because of the high transformation frequencies of E. coli K-12 relative to those...

...a strain which is restriction-negative (but modification-proficient) for the three restriction systems of **Salmonella** typhimurium, and also contains a mutation in galE resulting in higher transformation frequencies (for a description of restriction systems of **Salmonella** typhimurium, see Bullas et al., J. Bacteriol., 141:275 (1980)).

Plasmids were then inserted into attenuated **Salmonella** by transduction techniques. LB5010 containing the desired plasmid was grown in Luria broth (LB) to...

...0.7% agar. Phage were harvested and used to transduce plasmids into attenuated P22-sensitive **Salmonella** .

Electrotransfer Methods for Antigen Detection

Heterologous recombinant protein synthesis was detected in E. coli and **Salmonella** vaccine strain host cells by transblotting protein samples separated by polyacrylamide get electrophoresis onto nitrocellulose...

...the percent of plasmid-containing colonies.

RESULTS

Complementation of purA auxotrophy in E. coli and **Salmonella**

The product of the purA gene, adenylosuccinate synthetase (EC 6.3.4.4) catalyzes the...

...Chem. 263:19147-19153 (1988)). For construction of plasmids which complement either E. coli or **Salmonella** purA auxotrophies, plasmid pJS76 (obtained from John M. Smith, Seattle Biomedical Research Institute), was used...plasmid pPX3003, were transformed into S. dublin SL5653 or into S. typhimurium BB1231, derivatives of **Salmonella** vaccine strains harboring deletion mutations in the purA gene. **Salmonella** transformants were examined for presence of the anticipated plasmids by DNA analysis, and in the...

...particular plasmids, pPX3001 and pPX3003, are not likely to be optimally useful in a live **Salmonella** vaccine, since a combination of the purA and/or LT-B expression per se in the configuration above impedes the growth rate of the host **Salmonella** . In defined salts medium (M9) containing casamino acids, pPX3001/SL5653 and pPX3003/SL5653 had a...

...known that the high copy-number pUC vector plasmids are stable in E. coli and **Salmonella** strains. The observed instability of the purA derivatives of the pUC plasmids is probably due...

...gene expression tailored to be neutral to the in vivo growth properties of a live **Salmonella** vaccine strain, combined with genetic stability, should yield a maximally immunogenic vaccine configuration. Appropriate levels...

...the expressed antigen as well as the level to which it is expressed in the **Salmonella** vaccine strain. Several factors which may influence tolerance of the bacterial host strain to antigen...

...was stabilized by purA complementation and tailored to be neutral to the growth of a **Salmonella** vaccine strain, the effect of gene copy number on LT-B expression and tolerance by the **Salmonella** host was examined. This was examined under several conditions, one in which a lac promoter ...were flanked by the inverted repeat sequences. To create a suicide vector for use in **Salmonella** strains, the transposon carrying LT-B and kanamycin was crossed into a derivative of (lambda...

...67 (1990). The modified (lambda) phage carrying the LT-B transposon was used to infect **Salmonella** LB5010/F112, carrying an expressed (lambda) phage receptor. Since DNA of phage (lambda) does not replicate in **Salmonella** , clones selected for kanamycin-resistance result from transposition of the modified transposon onto the chromosome...

...lysates propagated on them were used to transduce the chromosomally located expression cassette into the **Salmonella** vaccine strain SL3261. Two independent SL3261 LT-B chromosomal integrants, resulting from transduction from two separate alleles, located in different loci in the **Salmonella** chromosome, were found to be stable in vitro and in vivo. Construction of a Low...

...compared with genes under control of weaker promoters such as the lac promoter. Further, because **Salmonella** strains are known not to be lysogenic for bacteriophage (lambda), expression of plasmid borne PL...

...copy-number of the vector plasmid, pPX3006 and will enhance immunogenicity of live attenuated recombinant **Salmonella** strains.

Several versions of the Plasmodium berghei circumsporozoite protein (CS) gene were stabilized in pPX3006...

...T-cells. Expression of the CS protein of P. berghei and P. falciparum in attenuated **Salmonella** and subsequent oral immunization has resulted in induction of specific CD+ cytotoxic T-cells recognizing...

...sporozoites.

Although protection against sporozoite challenge has been documented by vaccination with CS protein-expressing **Salmonella** , protection was incomplete, suggesting that adequate induction of immunity had not occurred. Further, this suggests that factors relating to CS protein expression in the attenuated **Salmonella** vaccine strain might be manipulated for enhanced immunogenicity. Such factors include, as mentioned above, gene...

...of the expressed protein. If, during the course of the transient infection by the attenuated **Salmonella** , a significant proportion of the immunizing bacteria lose plasmid due to segregation, effective immunization will...Expression of LT-B antigen was compared at different points in the growth cycle of **Salmonella** and was compared between gene integrated forms, high copy-number plasmid forms and low copy...

...containing the partitioning regions and functions associated with pSC101 are stable in E. coli and **Salmonella** under batch culture conditions. Although the par region of pSC101 is retained in pGD103 and...

...during batch growth, growth under nutrient-limiting conditions and during fermentation.

In Vivo Stabilization of **Salmonella** typhimurium Vaccine Strains by purA Complementation

To examine the stabilization of pSC101 plasmids by purA...invariably arise from the transposition of purA and LT-B to random locations on the **Salmonella** chromosome.

Strain Deposits

The following strains were deposited with the (ATCC), Rockville, Maryland, under the...

...SPECIFICATION by the pSC101 par locus. Other partition regions, such as a partition region from a **Salmonella** typhimurium virulence plasmid, have also been used successfully to stabilize cloning vehicles.

Although cloning vehicles...

...lead to plasmid stabilization. Complementation of a chromosomal mutation for aspartic semialdehyde dehydrogenase (asd) in **Salmonella** typhimurium or D-alanine racemase mutation (dal) in Bacillus subtilis, which each lead to faulty...CS fusion protein (pPX3007).

Figure 7 shows the results of in vivo stabilization studies of **Salmonella** typhimurium strains by purA complementation. S. typhimurium recovered from A: spleens, B: livers and C...

...purA gene product). The purA locus is especially important in the design of live attenuated **Salmonella** and related enteric vaccine vectors since the presence of purA mutations on the chromosome leads...

...or vitamin precursors (such as folic acid) which are dependent upon

aromatic compound biosynthesis. Thus, **Salmonella** typhimurium harboring deletion mutations in both aroA and in purA are ineffective in inducing a ...

...defects in aromatic biosynthetic genes (such as aroA) are effective in inducing immune responses. Although **Salmonella** harboring aroA mutations are able to replicate to a limited extent intracellularly, those containing additional...be used in the vaccine formulations of the invention include but are not limited to **Salmonella** spp., invasive E. coli (EIEC), and Shigella spp. In a preferred embodiment, invasive bacteria which reside in lymphoid tissues such as the spleen (e.g., **Salmonella** spp.) are used. Such bacteria can invade gut epithelial tissue and/or Peyer's patches...

...Cold Spring Harbor, New York), laboratory selection of natural mutations, etc. Methods for obtaining attenuated **Salmonella** strains which are non-reverting non-virulent auxotrophic mutants suitable for use as live vaccines...

...and 4,837,151 (issued June 6, 1989). A reliable method to achieve attenuation of **Salmonella** has also been described (S.K. Hoiseth, and B.A.D. Stocker, Nature, 291:238...

...53:47 (1982)) and can be used in a particular embodiment of the invention.

Attenuated **Salmonella** which can be used in the live vaccine formulations of the invention include but are not limited to those species listed in Table 1 below.

In specific embodiments, **Salmonella** bacteria that have been attenutated by chromosomal deletion of gene(s) for aromatic compound biosynthesis...

...equivalent to typhoid fever. ga1E mutants which can used include but are not limited to **Salmonella** typhi strains Ty21a (Germanier, Bacteria Vaccines, Academic Press, NY pp. 137-165) **Salmonella** typhimurium G30D, etc.

Expression of Gene Products and Uses

The invention also pertains to methods...encoding antigens derived from pathogenic bacterial, viral or parasitic sources can be introduced into attenuated **Salmonella** typhi for use as live vaccines in humans, to protect against, for example, typhoid fever...

...diseases and sexually transmitted diseases including AIDS. Alternatively, such genes can be introduced into other **Salmonella** capable of infecting animal species, e.g., S. dublin for use as live attenuated cattle...

...added at 100 (mu)g/ml.

Genetic Transformation and Transduction

Plasmids were introduced directly into **Salmonella** by electroporation with a BTX Transfector 100 with the 0.5 mm electrode and a...York (1982)). Plasmid constructions were isolated and characterized first in E. coli, before transferring to **Salmonella** spp., because of the high transformation frequencies of E. coli K-12 relative to those...

...a strain which is restriction-negative(but modification-proficient) for the three restriction systems of **Salmonella** typhimurium, and also contains a mutation in ga1E resulting in higher transformation frequencies (for a description of restriction systems of **Salmonella** typhimurium, see Bullas et al., J. Bacteriol., 141:275 (1980)).

Plasmids were then inserted into attenuated **Salmonella** by transduction techniques. LB5010 containing the desired plasmid was grown in Luria broth (LB) to...

...0.7% agar. Phage were harvested and used to transduce plasmids into attenuated P22-sensitive **Salmonella** .

Electrotransfer Methods for Antigen Detection

Heterologous recombinant protein synthesis was detected in E. coli and

Salmonella vaccine strain host cells by transblotting protein samples separated by polyacrylamide get electrophoresis onto nitrocellulose...

...the percent of plasmid-containing colonies.

RESULTS

Complementation of purA auxotrophy in E. coli and **Salmonella**

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...compared with genes under control of weaker promoters such as the lac promoter. Further, because **Salmonella** strains are known not to be lysogenic for bacteriophage (lambda), expression of plasmid borne PL...
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...sporozoites.

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...of the expressed protein. If, during the course of the transient infection by the attenuated **Salmonella** , a significant proportion of the immunizing bacteria lose plasmid due to segregation, effective immunization will...Expression of LT-B antigen was compared at different points in the growth cycle of **Salmonella** and was compared between gene integrated forms, high copy-number plasmid forms and low copy...

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...during batch growth, growth under nutrient-limiting conditions and during fermentation.

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To examine the stabilization of pSC101 plasmids by purA...invariably arise from the transposition of purA and LT-B to random locations on the **Salmonella** chromosome.

Strain Deposits

The following strains were deposited with the (ATCC), Rockville, Maryland, under the...

...CLAIMS pathogen.

11. The bacterium of claim 7 which is an enteroinvasive bacterium of the genus **Salmonella** , Shigella, Yersinia, Escherichia, Vibrio and Campylobacter.
12. A composition comprising the bacterium of any of...

...CLAIMS bacterium of any of Claims 6-8 which is an enteroinvasive bacterium of the genus **Salmonella** , Shigella, Yersinia, Escherichia, Vibrio and Campylobacter.

10. A composition comprising the bacterium of any of...of Claims 5 to 7 whereby said host bacteria are enteroinvasive bacteria of the genus **Salmonella** , Shigella, Yersinia, Escherichia, Vibrio or Campylobacter.
9. The method of any one of Claims 5...

...bacterium of any of Claims 6-8 which is an enteroinvasive bacterium of the genus **Salmonella** , Shigella, Yersinia, Escherichia, Vibrio and Campylobacter.

10. A composition comprising the bacterium of any of...

...CLAIMS Bakterium nach einem der Anspruche 6 bis 8, das ein entero-invasives Bakterium der Gattung **Salmonella** , Shigella, Yersinia, Escherichia, Vibrio und Campylobacter ist.

10. Zusammensetzung, umfassend das Bakterium nach einem der...einem der Anspruche 5 bis 7, worin das Wirtsbakterium ein entero-inyasives Bakterium der Gattung **Salmonella** , Shigella, Yersinia, Escherichia, Vibrio und Campylobacter ist.
9. Verfahren nach einem der Anspruche 5 bis...

...Bakterium nach einem der Anspruche 6 bis 8, das ein entero-invasives Bakterium der Gattung **Salmonella** , Shigella, Yersinia, Escherichia, Vibrio und Campylobacter ist.

10. Zusammensetzung, umfassend das Bakterium nach einem der...

...CLAIMS une quelconque des revendications 6 a 8 qui est une souche bacterienne enteroinvasive du genre **Salmonella** , Shigella, Yersinia, Escherichia, Vibrio et Campylobacter.

10. Composition comprenant la souche bacterienne selon l'une...5 a 7 dans lequel ledit hote bacterien est une souche bacterienne enteroinvasive du genre **Salmonella** , Shigella, Yersinia, Escherichia, Vibrio et Campylobacter.

9. Procede selon l'une quelconque des revendications 5...une quelconque des revendications 6 a 8 qui est une souche bacterienne enteroinvasive du genre **Salmonella** , Shigella, Yersinia, Escherichia, Vibrio et Campylobacter.

10. Composition comprenant la souche bacterienne selon l'une...

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File 411:DIALINDEX(R)

DIALINDEX(R)
   (c) 2004 The Dialog Corporation plc

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              1   349: PCT FULLTEXT_1979-2002/UB=20040415,UT=20040408
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N2            2    34: SciSearch(R) Cited Ref Sci_1990-2004/Apr W3
N3            1   342: Derwent Patents Citation Indx_1978-04/200420
N4            1   345: Inpadoc/Fam.& Legal Stat_1968-2004/UD=200417
N5            1   348: EUROPEAN PATENTS_1978-2004/Apr W02
N6            1   349: PCT FULLTEXT_1979-2002/UB=20040415,UT=20040408
N7            1   357: Derwent Biotech Res.__1982-2004/Apr W4
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  File  34:SciSearch(R) Cited Ref Sci 1990-2004/Apr W3
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File 342:Derwent Patents Citation Indx 1978-04/200420
(c) 2004 Thomson Derwent
File 345:Inpadoc/Fam.& Legal Stat 1968-2004/UD=200417
(c) 2004 EPO
***File 345: October 12, 2003 - changes to legal status now online.**
See HELP NEWS 345 for details.
File 348:EUROPEAN PATENTS 1978-2004/Apr W02
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File 349:PCT FULLTEXT 1979-2002/UB=20040415,UT=20040408
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(c) 2004 Thomson Derwent & ISI

Set	Items	Description

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	254	AU=FULGINITI ?
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1/3,KWIC/1 (Item 1 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

0012993775 BIOSIS NO.: 200100165614
Characterization and evaluation of the native and recombinantly-expressed 19.3 kDa Helicobacter **pylori ferritin**
AUTHOR: **Fulginiti J** (Reprint); Fiske M (Reprint); Caplan J (Reprint); Wetherell M (Reprint); Schmidt S (Reprint); Zhu D (Reprint); Dilts D (Reprint); Weidenborner P (Reprint); Belanger K (Reprint
AUTHOR ADDRESS: Wyeth Lederle Vaccines, West Henrietta, NY, USA**USA
JOURNAL: Gut 47 (Supplement 1): pA64 October, 2000 2000
MEDIUM: print
CONFERENCE/MEETING: XIIIth International Workshop on Gastroduodenal Pathology and Helicobacter pylori Rome, Italy October 11-14, 2000; 20001011
ISSN: 0017-5749
DOCUMENT TYPE: Meeting; Meeting Abstract
RECORD TYPE: Citation
LANGUAGE: English

Characterization and evaluation of the native and recombinantly-expressed 19.3 kDa Helicobacter **pylori ferritin**
AUTHOR: **Fulginiti J** ...
DESCRIPTORS:
ORGANISMS: **Helicobacter** pylori (Aerobic Helical or Vibrioid Gram-Negatives...

1/3,KWIC/2 (Item 2 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

0012992501 BIOSIS NO.: 200100164340
Identification of potential vaccine candidates from Helicobacter **pylori using proteomics technologies**
AUTHOR: Fiske M J (Reprint); Caplan J (Reprint); Wetherell M (Reprint); **Fulginiti J** (Reprint); Chakravarti D (Reprint
AUTHOR ADDRESS: Wyeth-Lederle Vaccines, West Henrietta, NY, USA**USA
JOURNAL: Gut 47 (Supplement 1): pA60 October, 2000 2000
MEDIUM: print
CONFERENCE/MEETING: XIIIth International Workshop on Gastroduodenal Pathology and Helicobacter pylori Rome, Italy October 11-14, 2000; 20001011
ISSN: 0017-5749
DOCUMENT TYPE: Meeting; Meeting Abstract
RECORD TYPE: Citation

LANGUAGE: English

Identification of potential vaccine candidates from Helicobacter **pylori using proteomics technologies**
...AUTHOR: **Fulginiti J**
DESCRIPTORS:
ORGANISMS: **Helicobacter** pylori (Aerobic Helical or Vibrioid Gram-Negatives...

1/3,KWIC/3 (Item 3 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

0011907919 BIOSIS NO.: 199900167579
Intragastric immunization with recombinant H. pylori urease formulated with attenuated cholera toxin elicits systemic, mucosal and protective immune responses in C57BL/6 mice
AUTHOR: Zhu D; Schmidt S; **Fulginiti J** ; Fiske M; Phillips E; Peek J; Green B; Eldridge J H
AUTHOR ADDRESS: Wyeth-Lederle Vaccines, West Henrietta, NY 14586, USA**USA
JOURNAL: FASEB Journal 13 (4 PART 1): pA291 March 12, 1999 1999
MEDIUM: print
CONFERENCE/MEETING: Annual Meeting of the Professional Research Scientists for Experimental Biology 99 Washington, D.C., USA April 17-21, 1999; 19990417
ISSN: 0892-6638
DOCUMENT TYPE: Meeting; Meeting Abstract
RECORD TYPE: Citation
LANGUAGE: English

...AUTHOR: **Fulginiti J**
DESCRIPTORS:
ORGANISMS: H. pylori { **Helicobacter** pylori} (Aerobic Helical or Vibrioid Gram-Negatives...

1/3,KWIC/4 (Item 1 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci

09228368 Genuine Article#: 368RK No. References: 0
Title: Characterization and evaluation of the native and recombinantly-expressed 19.3 kDa Helicobacter **pylori ferritin.**
Author(s): **Fulginiti J** ; Fiske M; Caplan J; Wetherell M; Schmidt S; Zhu D; Dilts D; Weidenborner P; Belanger K
Corporate Source: WYETH LEDERLE VACCINES,W HENRIETTA//NY/
Journal: GUT, 2000, V47, 1 (OCT), PA64-A64
ISSN: 0017-5749 Publication date: 20001000
Publisher: BRITISH MED JOURNAL PUBL GROUP, BRITISH MED ASSOC HOUSE, TAVISTOCK SQUARE, LONDON WC1H 9JR, ENGLAND
Language: English Document Type: MEETING ABSTRACT

Title: Characterization and evaluation of the native and recombinantly-expressed 19.3 kDa Helicobacter **pylori ferritin.**
Author(s): **Fulginiti J** ; Fiske M; Caplan J; Wetherell M; Schmidt S; Zhu D; Dilts D; Weidenborner P...

1/3,KWIC/5 (Item 2 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci

09228352 Genuine Article#: 368RK No. References: 0
Title: Identification of potential vaccine candidates from Helicobacter **pylori using proteomics technologies.**
Author(s): Fiske MJ; Caplan J; Wetherell M; **Fulginiti J** ; Chakravarti D
Corporate Source: WYETH LEDERLE VACCINES,W HENRIETTA//NY/
Journal: GUT, 2000, V47, 1 (OCT), PA60-A60

ISSN: 0017-5749 Publication date: 20001000
Publisher: BRITISH MED JOURNAL PUBL GROUP, BRITISH MED ASSOC HOUSE, TAVISTOCK SQUARE, LONDON WC1H 9JR, ENGLAND
Language: English Document Type: MEETING ABSTRACT

Title: Identification of potential vaccine candidates from Helicobacter **pylori using proteomics technologies.**
Author(s): Fiske MJ; Caplan J; Wetherell M; **Fulginiti J** ; Chakravarti D

1/3,KWIC/6 (Item 1 from file: 342)
DIALOG(R)File 342:Derwent Patents Citation Indx

04144476 WPI Acc No: 00-160674/14
Novel Helicobacter pylori antigens useful for diagnostic and therapeutic purposes -
Patent Assignee: (AMCY) AMERICAN CYANAMID CO
Author (Inventor): **FULGINITI J P** ; FISKE M J; DILTS D A
Patent (basic)
  Patent No Kind Date Examiner Field of Search
  WO 200000614 A2 000106 (BASIC)
Derwent Week (Basic): 0014
Priority Data: US 90851P (980626)
Applications: AU 9947189 (990625); WO 99US14375 (990625)
Designated States
  (National): AL; AM; AT; AU; AZ; BA; BB; BG; BR; BY; CA; CH; CN; CU; CZ; DE; DK; EE; ES; FI; GB; GE; GH; GM; HR; HU; ID; IL; IN; IS; JP; KE; KG ; KP; KR; KZ; LC; LK; LR; LS; LT; LU; LV; MD; MG; MK; MN; MW; MX; NO; NZ; PL; PT; RO; RU; SD; SE; SG; SI; SK; SL; TJ; TM; TR; TT; UA; UG; US ; UZ; VN; YU; ZA; ZW
  (Regional): AT; BE; CH; CY; DE; DK; EA; ES; FI; FR; GB; GH; GM; GR; IE; IT; KE; LS; LU; MC; MW; NL; OA; PT; SD; SE; SL; SZ; UG; ZW
Derwent Class: B04; D16
Int Pat Class: A61K-038/16; A61K-039/106; A61K-039/40; C07K-014/205; C07K-014/34; C07K-016/12; C12N-001/21; C12N-015/31
Number of Patents: 002
Number of Countries: 084
Number of Cited Patents: 004
Number of Cited Literature References: 002
Number of Citing Patents: 000

Author (Inventor): . **FULGINITI J P** ...

1/3,KWIC/7 (Item 1 from file: 345)
DIALOG(R)File 345:Inpadoc/Fam.& Legal Stat

15676264
Basic Patent (No,Kind,Date): CA 2329264 AA 20000106 <No. of Patents: 005>
NOVEL ANTIGENS OF HELICOBACTER PYLORI NOUVEAUX ANTIGENES D'HELIOBACTER PYLORI (English; French)
Patent Assignee: AMERICAN CYANAMID CO (US)
Author (Inventor): FISKE MICHAEL JAMES (US); DILTS DEBORAH ANN (US); FULGINITI JAMES PETER (US)
IPC: *C12N-015/31; A61K-039/106; C07K-016/12; A61K-038/16; C07K-014/205; C12N-001/21; C07K-014/34; A61K-039/40; C12N-015/62
Language of Document: English
Patent Family:

Patent No	Kind	Date	Applic No	Kind	Date	
AU 9947189	A1	20000117	AU 9947189	A	19990625	
CA 2329264	AA	20000106	CA 2329264	A	19990625	(BASIC)
WO 200000614	A2	20000106	WO 99US14375	A	19990625	
WO 200000614	A3	20000504	WO 99US14375	A	19990625	
WO 200000614	C2	20000330	WO 99US14375	A	19990625	

Priority Data (No,Kind,Date):
  US 90851 P 19980626
  WO 99US14375 W 19990625

Dialog File: Inpadoc/Fam.& Legal Stat_1968-2004/UD=200417

1/3,KWIC/8 (Item 1 from file: 348)
DIALOG(R)File 348:EUROPEAN PATENTS

01124761
NOVEL ANTIGENS OF $i(HELICOBACTER PYLORI)
ANTIGENE AUS HELICOBACTER PYLORI
NOUVEAUX ANTIGENES D'$i(HELIOBACTER PYLORI)
PATENT ASSIGNEE:
AMERICAN CYANAMID COMPANY, (212593), Five Giralda Farms, Madison, New Jersey 07940, (US), (Applicant designated States: all)
INVENTOR:
FULGINITI, James, Peter , 5180 Foster Road, Canadaigua, NY 14424, (US)
FISKE, Michael, James, 167 Wood Run, Rochester, NY 14612, (US)
DILTS, Deborah, Ann, 112 Country Downs Circle, Fairport, NY 14450, (US
PATENT (CC, No, Kind, Date): WO 200000614 000106
APPLICATION (CC, No, Date): EP 99930708 990625; WO 99US14375 990625
PRIORITY (CC, No, Date): US 90851 P 980626
DESIGNATED STATES: AT; BE; CH; CY; DE; DK; ES; FI; FR; GB; GR; IE; IT; LI; LU; MC; NL; PT; SE
INTERNATIONAL PATENT CLASS: C12N-015/31; C12N-001/21; C07K-014/205; C07K-016/12; A61K-038/16; A61K-039/40; C07K-014/34; C12N-015/62; A61K-039/106
LANGUAGE (Publication,Procedural,Application): English; English; English
NOVEL ANTIGENS OF $i(HELICOBACTER PYLORI)
ANTIGENE AUS HELICOBACTER PYLORI
INVENTOR:
FULGINITI, James, Peter ...

1/3,KWIC/9 (Item 1 from file: 349)
DIALOG(R)File 349:PCT FULLTEXT

00537241
NOVEL ANTIGENS OF i(HELICOBACTER PYLORI)
NOUVEAUX ANTIGENES D'i(HELIOBACTER PYLORI)
Patent Applicant/Assignee:
AMERICAN CYANAMID COMPANY,
FULGINITI James Peter,
FISKE Michael James,
DILTS Deborah Ann,
Inventor(s):
FULGINITI James Peter ,
FISKE Michael James,
DILTS Deborah Ann
Patent and Priority Information (Country, Number, Date):
Patent: WO 200000614 A2 20000106 (WO 0000614)
Application: WO 99US14375 19990625 (PCT/WO US9914375)
Priority Application: US 9890851 19980626
Designated States: AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES FI GB GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG US UZ VN YU ZA ZW GH GM KE LS MW SD SL SZ UG ZW AM AZ BY KG KZ MD RU TJ TM AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE BF BJ CF CG CI CM GA GN GW ML MR NE SN TD TG
Publication Language: English
Fulltext Word Count: 22776

NOVEL ANTIGENS OF i(HELICOBACTER PYLORI)
Inventor(s):
FULGINITI James Peter ...
Fulltext Availability:
Detailed Description

English Abstract

The present invention relates to novel nucleic acids and polypeptides relating to i(**Helicobacter** pylori), in particular novel i(H. pylori) bacterial surface proteins having molecular weights of approximately...

Detailed Description

00 PCT

NOVEL ANTIGENS of **HELICOBACTER** PYLORI

Field of the Invention

The present invention relates to novel nucleic acids and polypeptides relating to **HelicobacterpylorL** The nucleic acid sequences and polypeptides are useful for diagnostic and therapeutic purposes.

Back2round of the Invention

0 **Helicobacterpylori** (H. pylori) is a gram-negative, S-shaped, microaerophilic bacterium that was discovered and cultured...of the present invention have 5 diagnostic and therapeutic utility for H. pylori and other **Helicobacter** species. They can be used to detect the presence of H. pylori and other **Helicobacter** species in a sample, and to screen compounds for the ability to interfere with the...cholera toxin amino acid sequence. These may be added to, or conjugated with, the 0 **Helicobacter** antigenic composition. The same techniques can be applied to other molecules with mucosal adjuvant or...be covalently attached to molecules such as vitamin B12 which have specific gut receptors. The **Helicobacter** isolated polypeptides of this invention may also be incorporated into oily emulsions.

The **Helicobacter** isolated polypeptides of the present invention may be administered as the sole active immunogen in...

...composition. Alternatively, however, the antigenic, or vaccine, composition may include other active immunogens, including other **Helicobacter** antigens such as urease, lipopolysaccharide, Hsp60, VacA, CagA or catalase, as well as immunologically active...

...of this invention. Preferred embodiments relate to a method for the treatment or prevention of **Helicobacter** infection in a human comprising administering to a human an immunologically effective amount of an...

...infection.

Ideally, the treated individual will not exhibit the more serious clinical manifestations of the **Helicobacter** infection. The dosage amount can vary depending upon specific conditions of the individual. This amount...

...to a mammal a vaccine vector expressing at least one, or a mixture of isolated **Helicobacter** polypeptides of this invention, or an immunogenic fragment thereof. The isolated polypeptides of the present...a human, provides protection without inducing enhanced disease upon subsequent infection of the human with **Helicobacter** pathogen, such as H pylori.

Transfection-facilitating agents are known in the art.

The present...

...of this invention can be employed in a method for the treatment or prevention of **Helicobacter** infection in mammalian hosts, which comprises administration of an immunologically effective amount of antibody, specific...the nucleic acid molecule comprising such a sequence is capable of being expressed as a **Helicobacter** antigen as broadly described above.

The nucleotide sequence may have expression control sequences positioned adjacent...

...sequence having promoter sequences and initiator sequences and a nucleotide sequence which codes for a **Helicobacter** antigen, the nucleotide sequence being located Tto the promoter and initiator sequences. In yet another aspect, the invention provides a recombinant

DNA cloning vehicle capable of 6xpressing a **Helicobacter** antigen comprising an expression control sequence having promoter sequences and initiator sequences, and a nucleotide sequence which codes for a **Helicobacter** antigen, the nucleotide sequence being located 3' to the promoter and 1 0 initiator sequences...generally overwhelming.

1 9
In yet further aspects, there are provided fused polypeptides comprising a **Helicobacter** polypeptide of this invention and an additional polypeptide, for example a polypeptide coded for by...nucleic acid sequence described herein, one can generate synthetic polypeptides displaying the antigenicity of a **Helicobacter** isolated polypeptide of this invention. As used herein, the term "synthetic" means that the polypeptides...

...phase synthesis procedure.

Once recombinant DNA cloning vehicles and/or host cells expressing a desired **Helicobacter** polypeptide of this invention have been constructed by transforming or transfecting such cloning vehicles or host cells with plasmids containing the .

corresponding **Helicobacter** nucleic acid sequence, cloning vehicles or host cells are
2 0
cultured under conditions such...IO nucleotides are beneficial for providing stability and selectivity when testing a clinical sample for **Helicobacter** infection. A variety of known hybridization techniques and 0 systems can be employed for practice...obtained from T. Blanchard, Case Western Reserve University, Cleveland, OH.

2 4
.2 Culturing of **Helicobacter** strains. Cultures of H pylori and H felis were grown at 3 7'C on...protocol section) as the primary antibody in a Western blot against whole cell lysates of **Helicobacterpylori** and the recombinant 75, 77, and 79 kDa proteins. Conjugate 97-14 #14 generated a ...

1/3,KWIC/10 (Item 1 from file: 357)
DIALOG(R)File 357:Derwent Biotech Res.

0250832 DBR Accession No.: 2000-05322 PATENT
Novel Helicobacter **pylori antigens useful for diagnostic and therapeutic purposes - recombinant surface antigenic protein production via vector plasmid-mediated gene transfer and expression in bacterium for bacterium infection recombinant vaccine**
AUTHOR: **Fulginiti J P** ; Fiske M J; Dilts D A
CORPORATE SOURCE: Madison, NJ, USA.
PATENT ASSIGNEE: American-Cyanamid 2000
PATENT NUMBER: WO 200000614 PATENT DATE: 20000106 WPI ACCESSION NO.: 2000-160674 (2014)
PRIORITY APPLIC. NO.: US 90851 APPLIC. DATE: 19980626
NATIONAL APPLIC. NO.: WO 99US14375 APPLIC. DATE: 19990625
LANGUAGE: English

Novel Helicobacter **pylori antigens useful for diagnostic and therapeutic purposes**
AUTHOR: **Fulginiti J P** ; Fiske M J; Dilts D A
ABSTRACT: **Helicobacter** pylori surface protein antigenic proteins (I), with mol.wt. of 75,000 (Ia) (708 amino...
DESCRIPTORS: **Helicobacter** pylori recombinant surface antigenic protein prep., vector plasmid-mediated gene transfer, expression in bacterium, antibody...
?logoff hold

ORAL IMMUNIZATION OF MICE WITH LIVE ATTENUATED *SALMONELLA TYPHIMURIUM* EXPRESSING *HELICOBACTER PYLORI* UREASE.

J. Fulginiti, D. Zhu, S. Schmidt, P. Weidenborner, D. Lane, R. Deich and J.H. Eldridge. Lederle-Praxis Biologicals, West Henrietta, NY 14586

Attenuated *Salmonella* expressing foreign genes are attractive for use as oral vaccine carriers. We studied systemic and mucosal immune responses of BALB/c mice to *Helicobacter pylori* urease expressed in *Salmonella aroA* vaccine strain SL3261 following oral administration. Groups of eight-week old BALB/c mice were immunized intragastrically (IG) with 10^{10} *S. typhimurium aroA* transformed with plasmid pPX5024, which expresses *H. pylori* urease, on days 0, 2, 14 and 16. Control groups were immunized with 50 µg of purified native urease admixed with cholera toxin (CT), 10^6 SL3261/pPX5024 intraperitoneally (IP), or not vaccinated. Tissues were examined for the presence of the vaccine strain on days 1, 7 and 28. Two weeks after the last immunization, serum and bronchoalveolar washes (BAW) were collected to allow measurement of serum and mucosal IgA and IgG antibodies specific to urease in an ELISA. Results showed that plasmid-containing bacteria were recovered from the Peyer's patches, livers and spleens of IG immunized animals on all days examined. Both serum and BAW anti-urease antibodies were present after oral administration, but only serum antibodies were elicited by IP immunization. Mice which were orally immunized with 50 µg of purified native urease together with 10 µg of the adjuvant CT mounted both serum IgG and BAW IgA responses. However, oral immunization with *H. pylori* urease adjuvanted with CT induced a predominant IgG1 response, whereas *Salmonella* urease constructs preferentially induced an IgG2a response. Studies are underway to characterize the immune response which correlates with protection in the murine *Helicobacter felis* challenge model.

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FULGINITI James Peter,
FISKE Michael James,
DILTS Deborah Ann
Inventor(s):
FULGINITI James Peter,
FISKE Michael James,
DILTS Deborah Ann,
Patent and Priority Information (Country, Number, Date):
Patent: WO 200000614 A2 20000106 (WO 0000614)
Application: WO 99US14375 19990625 (PCT/WO US9914375)
Priority Application: US 9890851 19980626
Designated States: AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES FI GB GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG US UZ VN YU ZA ZW GH GM KE LS MW SD SL SZ UG ZW AM AZ BY KG KZ MD RU TJ TM AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE BF BJ CF CG CI CM GA GN GW ML MR NE SN TD TG
Publication Language: English
Fulltext Word Count: 22776

Patent Applicant/Assignee:
... **FULGINITI** James Peter
Fulltext Availability:
Detailed Description

Detailed Description
... strain ATCC 43 504; profile 4 is strain SS- I and profile 5 is a **urease** -negative strain.

5 Figure 4 is a graph depicting the bactericidal activity of anti-75... antigenic, or vaccine, composition may include other active immunogens, including other Helicobacter antigens such as **urease** , lipopolysaccharide, Hsp60, VacA, CagA or catalase, as well as immunologically active anti ens against other...

...immunogenic fragment as a foreign polypeptide. Particularly, bacteria that colonize the gastrointestinal tract, such as **Salmonella** , Shigella, Yersinia, Vibrio, Escherichia and BCG have been developed as vaccine vectors, and these and...for example, one will desire to contact and incubate the antisera-bound surface with a **urease** or peroxidaseconjugated anti-human IgG for a period of time and under conditions which favor...preferred diagnostic embodiments, one will 5 likely desire to employ an enzyme tag such as **urease** , alkaline phosphatase or peroxidase, instead of radioactive or other environmental undesirable reagents. In the case...using PBCC II 03 whole cell antigen as described above, partially purified protein mixture with **urease** and heat shock protein(s) and lipopolysaccharide (LPS) purified from PBCC II 03 cells. Secondary...on days 0, 2, 14, and 16 with I 00 @tg recombinant H. pylori (Hp) **urease** (rUre) or native Hp 75/77 kDa protein formulated with IO @tg cholera toxin (CT...

...comparable to, if not improved over, the reduction that is afforded by vaccination with recombinant **urease** admixed with CT.

3 2
Example 5 - Protein Analysis
Enzymatic Cleavage of the 75 kDa...day 0. For intragastric vaccinations, mice received native 100 @tg 75/77 kDa proteins, recombinant **urease** , or KLH admixed with CT on days 31, 33, 45, and 47. For subcutaneous vaccinations...

File 349:PCT FULLTEXT 1979-2002/UB=20040415,UT=20040408
(c) 2004 WIPO/Univentio

Set	Items	Description
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	3162	UREASE?
	10559	SALMONEL?
	28	FULGINITI
S1	2	UREASE? AND SALMONEL? AND FULGINITI

?t s1/3,kwic/all

1/3,KWIC/1

DIALOG(R)File 349:PCT FULLTEXT

00733255

19 KILODALTON PROTEIN OF HELICOBACTER PYLORI
PROTEINE DE 19 KILODALTONS PRODUITE PAR LA BACTERIE HELICOBACTER PYLORI

Patent Applicant/Assignee:
AMERICAN CYANAMID COMPANY, Five Giralda Farms, Madison, NJ 07940, US, US (Residence), US (Nationality), (For all designated states except: US)
Patent Applicant/Inventor:
FISKE Michael, 167 Wood Run, Rochester, NY 14612, US, US (Residence), US (Nationality), (Designated only for: US)
ZHU Duzhang, 50 Brittany Circle, Rochester, NY 14618, US, US (Residence), US (Nationality), (Designated only for: US)
FULGINTI James P, 5180 Foster Road, Canadaigua, NY 14424, US, US (Residence), US (Nationality), (Designated only for: US)
SCHMIDT Susan G, 455 Eastbrooke Lane, Rochester, NY 14618, US, US (Residence), US (Nationality), (Designated only for: US)
Legal Representative:
WEBSTER Darryl L, American Home Products Corporation, Patent Law Dept. 2B2, One Campus Drive, Parsippany, NJ 07054, US

Patent and Priority Information (Country, Number, Date):
Patent: WO 200046242 A2 20000810 (WO 0046242)
Application: WO 2000US2938 20000203 (PCT/WO US0002938)
Priority Application: US 99118631 19990204
Designated States: AE AL AM AT AU AZ BA BB BG BR BY CA CH CN CR CU CZ DE DK DM EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG US UZ VN YU ZA ZW
(EP) AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE
(OA) BF BJ CF CG CI CM GA GN GW ML MR NE SN TD TG
(AP) GH GM KE LS MW SD SL SZ TZ UG ZW
(EA) AM AZ BY KG KZ MD RU TJ TM
Publication Language: English
Filing Language: English
Fulltext Word Count: 9731

Fulltext Availability:
Detailed Description
Claims

Detailed Description
... however, the vaccine, composition may include other active immunogens, including other Helicobacter antigens such as **urease** , lipopolysaccharide, Hsp60, VacA, CagA or catalase, as well as immunologically active antigens against other pathogenic...immunogenic fragment as a foreign polypeptide. Particularly, bacteria that colonize the gastrointestinal tract, such as **Salmonella** , Shigella, Yersinia, Vibrio, Escherichia and BCG have been developed as vaccine vectors, and these and...for example, one will desire to contact and incubate the antisera-bound surface with a **urease** or peroxidase-conjugated anti-human IgG for a period of time and under conditions which...In preferred diagnostic embodiments, one will likely desire to employ an enzyme tag such as **urease** , alkaline phosphatase or peroxidase, instead of radioactive or other environmental undesirable

reagents. In the case...on days 0, 3, 14, and 16 with I 00 gg recombinant H. pylori (Hp) **urease** (rUre) or native Hp 19 kDa protein formulated with 1 0 [tg cholera toxin (CT...

Claim
... FlGw3
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<110> Fiske, Michael
Zhu, Duzhang
Schmidt, Susan G
Fulginiti , James P
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<130> 33537PCT
<140>
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<150> 60...

1/3,KWIC/2
DIALOG(R)File 349:PCT FULLTEXT

00537241
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Patent Applicant/Assignee:
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?logoff hold

28apr04 11:11:14 User228206 Session D2153.9
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$3.20 2 Types
$4.50 Estimated cost File349
$0.24 TELNET
$4.74 Estimated cost this search
$10.44 Estimated total session cost 2.457 DialUnits

Status: Signed Off. (2 minutes)

3425890 Supplier Number: 44769452 (USE FORMAT 7 FOR FULLTEXT)
Mucosal Delivery. Development of an Attenuated Salmonella **typhi Vaccine Strain for Use in Expression of Foreign Antigens**
Vaccine Weekly, pN/A
June 20, 1994
Language: English Record Type: Fulltext
Document Type: Newsletter; Trade
Word Count: 248

(USE FORMAT 7 FOR FULLTEXT)
Mucosal Delivery. Development of an Attenuated Salmonella **typhi Vaccine Strain for Use in Expression of Foreign Antigens**
TEXT:
AUTHORS: J. **Fulginiti** , R. Hazelo, P. Weidenborner and R. Brey. Lederle -Praxis Biologicals, Rochester, New York.
... 1994, in Las Vegas, Nevada, "In addition to their use as typhoid fever vaccines, attenuated **Salmonella** vaccines can be used to deliver foreign antigens to the intestinal mucosa. High-level expression...

...expression of foreign antigens from stable purA-complementing plasmids. Deletions in the purA gene of **Salmonella** result in mutants which require supplementation with adenine for growth and which are attenuated in virulence. Complementation of purA in vaccine strains of **Salmonella** can be used instead of antibiotics to select for transformants. The B subunit of the...